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PHYSIOLOGICAL REVIEWS

VOL. XI JULY, 1931 No. 3

THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNITY

R. H. JAFFÉ

From the Department of Pathology, Cook County Hospital, Chicago

In his studies on natural and acquired immunity, Metschnikoff emphasized the great importance of phagocytosis for the defense reactions of the body against microbic invasion. Metschnikoff distinguished two types of phagocytic cells, namely, the leukocytes of the circulating blood which he called microphages, and large cells with oval nuclei more difficult to stain than the nuclei of the leukocytes. This latter type of cells, derived from fixed tissue cells, were called macrophages. Shortly after Metschnikoff's discoveries Wyssokowitsch described that bacteria and spores of fungi injected into the blood stream of rabbits were taken up by the vascular endothelial cells and thus removed from the blood.

In the following years of rapid development of immunology stimulated by Behring's and Kitasato's discovery of the diphtheria antitoxin, relatively little significance was attributed to phagocytosis except for the intake of bacteria by leukocytes, a process which was found to be influenced by substances of the serum belonging to the so-called antibodies.

In the meantime histological studies had revealed that in the loose connective tissue there occurred, in addition to the fibril-producing connective tissue cells, branched elements with a more abundant cytoplasm, which arranged themselves especially about the small blood vessels. These cells responded to slight irritation by withdrawing their processes, assuming a round shape and displaying amoeboid movements. To these cells the study of which was greatly advanced by the introduction of the acid vital stains, different names were given (clasmatocytes, rhagiocrine cells, resting wandering cells, polyblasts, adventitial cells, pyrrhol cells). Vital stains also showed that these cells were not restricted to the loose connective tissue but that similar cells were present

in certain organs, notably liver, spleen, lymph nodes and bone marrow where they lined the capillaries and formed the reticulum (Ribbert, Goldmann, Kiyono, etc.). Later it was found that all these cells showed a great affinity not only to the acid vital stains but to electronegative colloids in general, to finest suspensions and to lipoids.

Aschoff united the cells so widely distributed throughout the body under the name: reticulo-endothelial system. The reticulum and endothelial cells of the lymph nodes, spleen and bone marrow, the Kupffer cells of the liver and the endothelial cells of the capillaries of the inner portion of the suprarenal cortex form the reticulo-endothelial system in the narrower sense while the reticulo-endothelial system in the wider sense also includes the perivascular cells (adventitial cells). The mobile cells derived from the reticulo-endothelial system are called free histocytes.

After the morphology of the cells had been well established the question of their functional qualities aroused wide attention. Numerous histological observations suggested that the reticulo-endothelial cells were endowed with many important functions. They take a very active part in the inflammatory and reparatory processes, engulf and dissolve erythrocytes, retaining the iron in the form of granules. Local or generalized disturbances of the fat metabolism lead to a piling up in their cytoplasm of fatty substances.

The functional significance of an organ or tissue is revealed by the disturbances which result from its removal. The wide distribution of the reticulo-endothelial cells throughout the body makes their elimination a great problem. Extirpation of the spleen reduces somewhat the number of these cells but the greater part of them remains in the body. In addition, the spleen contains many other cells and its loss is soon compensated by a hyperplasia of the other reticulo-endothelial cells. While studying the place of bilirubin formation, Aschoff's pupil Lepehne, and Eppinger thought that it might be possible to eliminate functionally the reticulo-endothelial cells by filling them with colloidal metals. Most of the later functional studies on the reticulo-endothelial system, especially those concerning their rôle in immunity, were carried out with the aid of this so-called blocking method and a description and criticism of this method, therefore, may precede the discussion of the relation between immunity and reticulo-endothelial system.

THE METHOD OF BLOCKING THE RETICULO-ENDOTHELIAL SYSTEM. Is it possible, by injecting electronegative colloids or fine suspensions of carbon, to stuff the reticulo-endothelial cells with these substances so

that they are unable to function? Many investigators (Bieling and Isaac, Cannon, Baer, Sullivan and Webster, Cionini, Gay and Clark, Isaacs, Jungeblut and Berlot, Kobayashi and Shiwotsu, Kritschewski, Neufeld and Meyer, Roberts, Siegmund, Stewart and Parker, Vannucci, and others) accept this possibility although most of them admit that it is rather a temporary depression than a complete elimination of the function. Even among these authors the results are not uniform. Some of the blocking substances have been found to be more efficient than others. The same substance may depress certain functions and increase other ones, or not affect them at all (Gay and Clark, Jelin, Stewart and Parker, and others). Numerous investigators were unable to see any depressing effect of the so-called blockade (Alymow and Gurwitsch, Benassi, Bruyhnoghe and Collon, Fraenkel and Grueneberg, Frei and Weidlich, Howell and Tower, Howell and Beverley, Leontjeff-Warschamow, Lewis and Loomis, Rosenthal and Fischer, Smith, Simitch, Standenath, Weiss and Kunze). An occasional increase of the functions is mentioned by Benassi, Lewis and Loomis, Rosenthal and Fischer, Seiffert, Standenath and Weiss and Kunze.

This discrepancy between the results is readily understood if one keeps in mind that the method of blocking the histocytes allows an unlimited number of variations. Practically everyone of the authors mentioned had his own way of attempting the functional depression and it seems, therefore, worth while to discuss briefly the different factors which influence the effect of the storage.

1. *Animal species.* The blockade experiments were chiefly done on dogs, rabbits, guinea pigs, mice and rats, to a lesser extent on birds and cold blooded animals. The most suitable animals apparently are mice, rabbits and birds, while in dogs difficulties may be encountered.

2. *Number of animals used.* Since considerable individual variations exist among the animals of a species a large number of animals has to be used and it is not permissible to draw conclusions from an experiment on two or three animals. This has been justly emphasized by Cannon, Baer, Sullivan and Webster.

3. *Age of animals.* Adult animals only should be used because young animals are poor antibody producers (Freund, Friedberger and Simmel, Moll, Kligler and Olitzki).

4. *Kind of blocking substance.* Of the acid vital stains lithium carmin should not be used for blockade experiments since it is too toxic. But also the other vital stains, trypan blue, pyrrhol blue, isamin-blue, etc., cannot be called indifferent. When solutions of these dyes are injected

subcutaneously or into a body cavity they cause a distinct though slight inflammatory reaction (Klinge, see also Dustin). Colloid metals too are not harmless and in addition, the protecting colloid has to be taken into consideration, especially if it is a protein. Saccharated iron oxide is less toxic than most of the other colloid metals (Pfeiffer and Boerner-Patzelt). India ink has been used very widely. As advantages of India ink are mentioned that the carbon particles are inert and stay in the cells longer than any of the other substances. But most of the fine suspensions of carbon (India ink, Chinese ink, etc.) too, contain a protecting colloid. The coal reaches chiefly the litoral cells, that means those cells which are in intimate contact with the blood- or lymph-stream while the adventitial cells are hardly stained.

5. *Route of introduction of the blocking substance.* An even and quick distribution of the blocking substance is accomplished only by injecting it into the blood stream. Subcutaneous or intra-abdominal injections are by far less satisfactory.

6. *Amount of blocking substance.* This factor has often been neglected, too small amounts of dye, colloidal metal or ink having been used. It is not surprising that these small amounts often exert a stimulating influence since vital staining seems to follow the Arndt-Schulze law in that small amounts stimulate and large amounts depress the function of the storing cells. Blocking dogs with collargol and injecting later colloidal iron, Leites and Raibow found that at first there was a decrease of the storage of colloidal iron, to be followed by an increase. It is surprising that according to their experience the activation of the reticulo-endothelial system is most marked after very intensive blockade.

7. *Number of injections.* In most of the experiments the blocking substance was given repeatedly. Sometimes very many injections were used and were spread over weeks in the hope of obtaining a very effective blockade. Because of the quick regeneration by which histocytes compensate any loss, this prolonged vital staining is of little value. Unless the injections are made in intervals of a few hours the dye stuffed histocytes are substituted for by new ones before the next injection is given. This may go on indefinitely and reminds one of filling a jar with a hole in the bottom.

8. *Combination with splenectomy.* In many of the experiments the blockade has been combined with splenectomy. Removal of the spleen eliminates part of the reticulo-endothelial system and the injected dye is distributed over a smaller number of cells. The significance of the loss of the spleen varies with the animal species used. For the rat the

loss of the spleen means more than it does for the rabbit because in the rat the spleen is relatively five times larger than in the rabbit (Krumbhaar and Musser). The injections of the blocking substance have to start the day after the operation lest the effect of the splenectomy be compensated by proliferation of the remaining histocytes.

9. *Excretion of the blocking substance.* Not all of the vital stain or colloidal metal is deposited in the histocytes, part of it being excreted before it has been taken up by these cells. The vital stains leave the body through the liver and the kidneys, colloidal iron chiefly through the large intestine. The importance of the excretion has been recently pointed out by Victor, Van Buren and Smith who have found that the slowing of the elimination of brilliant vital red from the blood stream which is caused by intravenous injections of India ink is not due to the filling of the reticulo-endothelial cells with carbon but to an inhibitory action of the ink upon the excretion of the dye through the bile. Preliminary experiments seem to suggest that it is not the carbon content of the ink which is responsible for this inhibition.

10. *Relation between blockade and functional test of the reticulo-endothelial system.* The rapidity with which the reticulo-endothelial cells compensate any loss has already been mentioned. This compensation is accomplished by an increase in size of the cells and especially by an increase in number. Victor, Van Buren and Smith also speak of an extension of the group of phagocytic cells in so far as, for instance, the fibrocytes take over some of their functions. I do not think that this extension is of much importance since even after most intensive vital staining only very little dye is stored by the fibrocytes and common vascular endothelial cells.

The quick regeneration of the reticulo-endothelial cells makes it necessary that the testing of their function is done shortly after the blocking injection and the lapse of time should not exceed six hours. When one waits longer the results are irregular. In experiments on the response to antigenic substances the amount of antigen is of great importance and too large doses of the antigen have often caused an obscuring of the results (Cannon, Baer, Sullivan and Webster). When several injections of antigen are given each of these injections should be preceded by an injection of the blocking substance. The blocking should always be done before the administration of the antigen and no result can be expected if the experiment is carried out in the opposite way.

11. *Mechanism of vital staining.* Vital staining is an intricate process which consists of several phases (Von Moellendorff, de Haan, and others).

Adsorbed by the proteins of the body fluids the stain enters the cell until a certain ratio between the concentration of the dye in the cell and surrounding fluids is reached. When the concentration of dye in the fluids increases more dye enters the cell (Smith). At first there is a diffuse inhibition of the dye by the protoplasm of the cell which stimulates a series of intracellular changes. These changes result in separation of the protoplasm from the irritating dye. The dye is segregated into extraplasmatic vacuoles, and by concentration, dehydration, and precipitation it is flocculated in the form of granules. With the formation of the granules the process comes to a stand still and the cell returns to the resting stage. The storage of the dye does not affect the vital parts of the cells (Von Moellendorff) and in the dye filled cells sufficient cytoplasm is left to carry on its metabolic processes. The cell also is able to take in more foreign material of the same or of another kind. It is, therefore, not surprising that several different dyes can be stored by the same cell (Smith and many others). I would recall in this connection the heart failure cells of the lung in which the iron pigment of the disintegrating blood cells accumulates especially about the coal granules and of the simultaneous occurrence of coal pigment and tubercle bacilli in some of the macrophages in tuberculosis of the lung.

The most marked effect of the blockade is to be expected during the period of intracellular segregation of the foreign substance when the cell uses all its forces to get rid of it. This has been well illustrated by Klinge's interesting studies on the effect of the blockade on the Arthus phenomenon.

From this discussion of the process of vital staining it will be clear that the functional depression of the histocytes is only a very transient one. I do not believe that there exists a physiological blockade by the endogenous and exogenous pigments as it has been described by Bratiano and Llombart.

Jungeblut and Berlot tested the cellular vitality of liver and spleen following blockade with ink. Using the cell respiration as an indicator they found that the respiration is depressed within one hour after the injection. The depression lasts for eight hours and is followed by recovery. These experiments are, however, not clear cut because the suspensions of liver and spleen contain too many different kinds of cells.

12. *Effect of the blocking substances on tissues other than the reticulo-endothelial system.* Although the vital stains are stored chiefly by the histocytes small amounts of them appear also in other cells, in particular

in parenchymatous cells. Many of the acid vital stains can also be found in the liver cells and epithelial cells of the convoluted tubuli of the kidney and after injecting saccharated iron one can see iron granules in the epithelium of the cecum. After long continued injections even the carbon particles of India ink may finally enter the liver cells and the cells of the suprarenal cortex. Furthermore one should not forget the effect of the injected substance upon the blood itself, its cellular elements as well as its physical properties and chemical composition. With regard to the latter question little work has so far been done. Seiffert states that splenectomy and blockade causes in guinea pigs an increase of the sedimentation rate of the erythrocytes and an increase in number of the stalagmotetric drops of the serum, and Natali found in blocked rabbits a prolong action of the coagulation time of the blood.

Summarizing the discussion on the reticulo-endothelial blockade it can be said that, under certain conditions, a temporary functional depression of the histocyte is possible. This depression is most marked during the period of intracellular segregation of the electronegative colloid while after completed storage the function is affected very little or not at all. The great regenerative ability of the histocytes is of the utmost importance. The neglect of the time factor, the toxicity or the small amounts of the blocking substances, the insufficient number of animals used and the excess of antigenic substance make many of the experiments on reticulo-endothelial blockade practically worthless.

RETICULO-ENDOTHELIUM AND ANTIGEN. If immunity is a function of the reticulo-endothelial cells, intimate relation between the antigens and these cells is to be expected. The fate of the formed antigens, animate as well as non animate, can be easily followed by microscopic observation and numerous experiments have shown that these formed antigens are quickly taken up by the reticulo-endothelium and that in the living organism the macrophagocytosis by the fixed histocytes by far surpasses the microphagocytosis by the circulating leukocytes. An excellent review of the publications dealing with this question has been recently given by Jungeblut. The problem becomes more complicated when one tries to investigate what happens to the liquid antigens, in particular the toxins and foreign proteins, after they have been injected into the body. Since there are no methods available to demonstrate the liquid antigens in the cells their fate can only be suggested. It has been shown that the spleen of the mouse is able to store within a few minutes large amounts of antigen (Beiling). The storing power of the guinea pig's spleen is much less marked. The spleen also adsorbs and

keeps for a certain period of time diphtheria and tetanus toxin and liver and adrenal take part in this storage (Bieling, Bieling and Gottschalk).

Histological studies show that foreign proteins stimulate the reticulo-endothelial cells. The cells swell, proliferate, their cytoplasm becomes more basophilic, they segregate fluid material in vacuoles and display an increased storage of acid vital stains (Siegmund, Epstein and others), changes which suggest that these cells may take in some of the foreign protein.

Forster, Opie and others have found that antigenic proteins, after an initial quick disappearance, remain in the blood in small quantities over a longer period of time. According to Collon, blockade does not interfere with the elimination of heterologous serum and Roberts observed that India ink blockade of rabbits increases by approximately 50 per cent the rate of disappearance of human serum from the blood stream. Roberts explains his findings by an increase in permeability of the endothelial membranes which allows the cells to take up the foreign protein with greater avidity.

The conditions, however, become much more intricate when we study the finer mechanism of the elimination and denaturation of the foreign protein. Manwaring speaks of an homologenization of the injected antigen with the animal tissue. The power of the retained foreign protein to produce an anaphylactic shock in another sensitized animal decreases rapidly and by the fourth day no undenatured protein can be demonstrated by means of sensitizing or calling forth an anaphylacic shock. The precipitinogenic properties persist longer but they too later become denatured as shown by a distorted precipitin graph (Manwaring). Endothelial blockade with ink has a distinct influence on the anaphylactic denaturation process and half of the injected protein is still active as late as nine days after the injection (Boone). This may indicate that the reticulo-endothelium has something to do with the homologenization of the antigen. Manwaring's very important investigations seem to open a new and wide field for additional information concerning the transformation of the antigen in the animal body, information which may help in clearing the still uncertain question of the relations between liquid antigens and reticulo-endothelium. For the time being, it is chiefly from the reactions to the antigens, from the specific antibodies, that such relations can be deducted.

The extensive work on immunology of recent years has forced a complete revison of our conception of the different processes which comprise immunity. The sharp differentiation between phagocytosis and action

of antibodies which formerly has caused so much controversy no longer is justified and it is perhaps the most important outcome of all the studies on immunity and reticulo-endothelial system that both the intracellular and extracellular destruction of foreign material are considered as expressions of the same cellular reactions of the host (Bieling, Taliaferro).

RETICULO-ENDOTHELIUM AND NATURAL RESISTANCE. Among the different factors which determine the natural resistance of the body to microorganisms, macrophagocytosis is one of the most important. Saprophytic and non-pathogenic bacteria brought into the blood stream are quickly taken up and destroyed by the reticulo-endothelial cells. Jelin injected different saprophytic bacteria into the abdominal cavity of rabbits and found that some of them (staphylococcus albus, bacillus mycoides roseus) are rapidly engulfed by the phagocytic cells of the peritoneal lining. The intracellular digestion of the bacteria is associated with the excretion of dissolving enzymes into the fluid of the abdominal cavity which thus acquires bactericidal properties. Other bacteria (B. anthracoides) are disposed of by preëxistent bactericidal substances in the abdominal fluid and phagocytosis by sessile or mobilized histocytes is of lesser significance. A phagocytic phenomenon which has been frequently studied since Metschnikoff's time is the natural resistance of certain animals against anthrax bacilli. Bardach made dogs susceptible to an infection with these bacilli by removing the spleen and injecting powered wood charcoal. Singer broke the natural resistance of hens against anthrax bacilli by blocking the reticulo-endothelial cells with ink. The susceptibility of rabbits, mice and guinea pigs to anthrax was explained by Metschnikoff on the basis of a deficient phagocytosis. Rabbits are highly susceptible to anthrax when the bacilli are injected through the skin (Besredka), but they are resistant when the bacilli are injected intratracheally because they are phagocyted and destroyed by the fixed macrophages of the lung (Fried). Collon's experiments on the resistance of frogs, hens and dogs to infections with anthrax bacilli gave different results. This author was unable to render the animals susceptible by blocking them with saccharated iron, carmin or starch. He also saw no effect of the blockade on the resistance of guinea pigs to the bacilli of hog erysipelas. Frei and Weidlich believe that the defense of white mice against these bacilli depends chiefly upon the reticulo-endothelial system.

Strains of bacillus pestis of so low a virulence that they do not cause any reaction in normal guinea pigs and rabbits kill these animals after they have been blocked with lithion carmin or trypan blue. In passing

through the blocked animals the virulence of the bacteria is somewhat increased (Fialho and Bacheco).

Frogs cannot be infected with staphylococcus aureus. Pickoff found that the Kupffer cells of the frog quickly take up the cocci when injected into the blood stream. Pigeons are resistant to pneumococci because their reticulo-endothelium destroys them (Kyes, Berry and Melick). In the serum of animals non-susceptible to pneumococcic infection there are, however, antipneumococcic substances (Bull, Bull and McKee, Robertson and Sia). Sera of normal dogs clear suspensions of highly virulent pneumococci. The significance of these pneumococcidal substances remains to be proved. In early stages of lobar pneumonia, Robertson, Terrel, Graeser and Cornwell did not find a decrease of the pneumococcidal action of the serum against homologous strains of pneumococci. Lobar pneumonia, therefore, can occur in the presence of the normal pneumococci killing substances of the serum. Robertson, Terrel, Graeser, and Cornwell are compelled to assume local changes in the lung permitting the pneumococci to multiply and protecting them from the pneumococcidal action of the serum. Do these experiments not point to the great importance of the defense mechanism offered by the reticulo-endothelial cells?

Schürer-Waldheim discusses the possibility of increasing the resistance of the body to infections by radiating the spleen. Kaufmann's cantharidin blister method enables one to study during life the changes in the reactivity of the blood forming tissues. Increase in the reactivity of the reticulo-endothelial system is shown by a high monocyte count of the blister content. In normal individuals the blister fluid contains in average 4.9 per cent monocytes. After radiation of the spleen the monocyte count goes up to 17.6 per cent.

With regard to the resistance against spirochetes the experiments of Pentschew may be mentioned. Injected into the abdominal cavity of young rabbits spirochaeta gallinarum appears in the blood for a very short period of time and then disappears (Levaditi). The same holds true of mice (Deutz). When blocked with saccharated iron or colloidal bismuth the spirochetosis of the blood of the mouse is markedly increased. Splenectomy has no effect. Experiments of Jungeblut on transferring human tertial malaria into splenectomized or blocked mice had no results.

According to Jungeblut, natural resistance against toxins is independent of the reticulo-endothelial system. Especially the antitoxic immunity demonstrates the complexity of the natural resistance and shows the

importance of the affinity of the virus and its products to the tissues of the host. In the serum of the non-susceptible rat, diphtheria toxin is circulating freely (Coca, Russel and Baughman) and the erythrocytes of the rat do not absorb the toxin while the erythrocytes of the very susceptible guinea pig (Starsky) do so. Gruber speaks of three lines of defense, namely, the intact skin to which I would add the intact mucous membranes, the tissue fluids and the macrophages. In this connection it may be remembered that the resistance against tuberculosis seems to be influenced by lipolytic enzymes of the blood (Metalnikow).

RETICULO-ENDOTHELIAL SYSTEM AND INFECTION. When the reticulo-endothelial cells are not able to free the body from the invading micro-organisms the infection takes. There may be an initial phagocytosis of virulent microorganisms circulating in the blood but the sterilization of the blood is only temporary (Bieling, Bail and Singer). While in the case of avirulent bacteria phagocytosis is identical with their definitive elimination virulent bacteria overcome the macrophagocytosis, be it by their being protected by capsules or by their excreting cell destroying substances. Hence they return into the blood and increase until the animal dies (Bail, Oerskov, Jensen and Kobayashi). Bull describes that virulent pneumococci injected intravenously decrease in number, and that in fatal infections the decrease is followed by an increase with death from septicemia. The avirulent R form of the pneumococcus is rapidly taken out of the blood stream of the rabbit while slightly virulent S forms first decrease and then increase in number (Tillelt). Injecting pneumococci into the bone marrow of susceptible normal animals Singer observed a slight phagocytosis by the reticulum cells which, however, was soon overshadowed by an intense intra- and extracellular proliferation of the cocci.

The infection with streptococci seems to follow similar laws. Unchecked streptococcus septicemia results from a lack of activation of the histocytes, in particular of the endothelial cells of the liver. In sepsis lenta, which indicates a high resistance of the body, there is a swelling and proliferation of the Kupffer cells (Siegmund). According to Louros and Scheyer, the outcome of a streptococcus sepsis depends upon the reactivity of the reticulo-endothelial system. In rapidly fatal streptococcus infections of the mouse the reticulo-endothelial cells are anergic and extensive necrosis in liver and spleen develops. Defense reactions manifest themselves in a swelling and proliferation of the histocytes, notably in liver and spleen. This defense reaction may be successful and the animal survives or it may break down and the animal

dies from a delayed septicemia. In human puerperal fever the cases with very acute septicemia and death within a few days and those who die from peritonitis represent the anergic type. In the thrombophlebitic group, which may live for several weeks, there is an initial reaction of the reticulo-endothelial cells with successive exhaustion. The more marked is phagocytosis and hyperplasia of the histocytes the longer the duration of the disease (Scheyer). Based upon these observations Louros and Scheyer attempted a more rational therapy of streptococcus septicemia by stimulating the reticulo-endothelial cells.

Siegmund's and Louros and Scheyer's investigations are of particular interest with regard to subacute endocarditis which is a special form of sepsis lenta. Cases of subacute endocarditis reveal a marked activation of the histocytes throughout the body and the formation of intimal granulomas (Siegmund). It is very difficult to demonstrate streptococci in the intimal nodules and in the swollen and proliferated histocytes of the liver, spleen and bone marrow. If, however, in the terminal stages a complete breaking down of the defense reactions takes place the streptococci multiply rapidly and cause the formation of abscesses which are considered rare in subacute endocarditis. Such abscesses may be found in the kidneys, spleen and brain, and especially in the brain the replacement of the glial-histocytic nodules by streptococci filled abscesses is very striking.

There is also the possibility that from the very beginning of the infection the reticulo-endothelial cells cannot engulf the bacteria. Physical properties of the surface of the microorganisms may render them unaccessible to the macrophages, or the bacteria may excrete substances injurious to the histocytes (aggressins) (Bail), virulins (Rosenow)), or the histocytes may be altered by products of an abnormal metabolism (Singer).

In this connection I want to mention that the presence of microorganisms in the histocytes does not necessarily mean phagocytosis and that the parasites may actively invade the cells making them their host. Thus, leishmaniosis is a disease of the recticulo-endothelial system. Hammerschmidt cultivated a diphtheroid bacillus from the brain of a dog dead from rabies which infected all the endothelial cells of the mouse.

It will be interesting to investigate how depression of the functional activity of the reticulo-endothelial cells by blockade or reduction of their number by splenectomy influences the course of infections. Streptococci kill splenectomized or trypan blue blocked mice much faster than normal controls (Meersohn, Tsuda, Louros and Scheyer; see, how-

ever, the negative results of Bieling). Siegmund observed that the resistance of mice against pneumococci was increased by injecting collargol, ink or carmine. Murray working with meningococci, found their virulence increased when injected into blocked mice. Jelin explains the greater resistance of blocked rabbits against intestinal infection with typhoid bacilli on the base of an inhibition of the intracellular destruction of the bacteria. The controls die because the histocytes liberate the endotoxins of the phagocyted microörganisms. The blocked rabbit survives though the bacilli which are not phagocyted remain and increase in the blood stream. In the infection of the mouse with the Breslau bacillus blockade and splenectomy prevent the initial sterilization of the blood and the animals die quicker (Oerskow, Jenen and Kobayashi). Intraperitoneal blockade aggravates a subsequent parathyroid B infection of white mice (Elbert). Intraperitoneal blocking of rabbits and guinea pigs by India ink or colloidal iron injections four hours before an intraperitoneal injection of B. mallei shortens markedly the incubation (Kostyorko). Schwienhorst studied the effect of roentgen rays on the phagocytic activity of the reticulo-endothelial cells and observed that an inhibition of their activity was associated with a higher mortality. Reimann followed the effect of injections of colloidal substances on the experimental typhus infection of guinea pigs and found that these injections prolonged the incubation time and shortened or diminished the febrile reaction.

Of the chronic bacillary infections tuberculosis shows the most marked histocytic reaction. Most of the investigators did not see any effect of blockade on the experimental infection of the guinea pig and rabbit (Jaffé, Jaffé and Levinson, Jungeblut, Seiffert, Kostyorko). Blockade also does not increase the virulence of the Calmette-Guérin strain of tubercle bacilli (Seiffert). Splenectomy shortly before the infection decreases the resistance of the mouse (Lewis and Margot, Murphy and Ellis). Kellert noticed an increased susceptibility of the guinea pig to tuberculosis after radiation. Beitzke mentioned a relatively light course of tuberculosis in vitally stained guinea pigs and Corper, Stary and Lurie saw a favorable influence of India ink injections in mild and protracted infections of the rabbit. Wedekind considers the possibility of stimulating the function of the reticulo-endothelial system in human cases of tuberculosis by injecting finest suspensions of carbon. The unfavorable effect of insulin medication in tuberculosis is perhaps due to the depression of the storage function of the histocytes by the insulin.

Amako, Jungeblut, Kritschewski and Rubinstein, Lisgunowa and

Butjagina and Rubinstein report on the deleterious effect of splenectomy and blockade on the experimental infection of mice, rabbits, rats and guinea pigs with the different strains of the spirochete of relapsing fever. Especially removal of the spleen greatly increases the mortality (Lisgunowa and Butjagina, Kritschewski and Rubinstein; see also the earlier experiments by Metschnikoff, Soudakewitsch (monkeys) and others). Similar experiments by Feldt and Schott, Bruynoghe and Collon and Seiffert did not show the marked differences between blocked and normal animals described by the other authors, discrepancies which are explained by Rubinstein on the base of a difference in the virulence of the spirochete strains used. In splenectomized chicken spirocheta gallinarum increases more rapidly than in normal animals (Lannoy and Levy-Bruhl). Infections with syphilis spirochete (Jungeblut) and the Weil spirochete (Zimmermann, Seiffert, Tschikow and Rubinstein) do not seem to be influenced by blockade or splenectomy.

Jungeblut does not think that the reticulo-endothelial system is concerned with the defense mechanism in trypanosomiasis if these protozoa are very virulent. An increase of the severity of the infection with trypanosomes after splenectomy is described by Kritschewski and Regendanz, Nieschulz and Wawo-Roentoe, Krumbhaar and Amako. A strain of trypanosomes the virulence of which had been much diminished by a subtherapeutic dose of neosilversalvarsan could be kept by Seiffert only in splenectomized mice. Splenectomy and blockade do not affect the infection with schizotryanum cruzi (Nieschulz, Kritschewski) while the resistance against piroplasmosis is decidedly diminished (Gonder and Rodenwald, Kikuth, Regendanz and Kikuth, Jimenez de Asua, Dios, Zuccarini and Kuhn).

There are a number of experiments which suggest intimate relations between the vaccinia virus and the reticulo-endothelial system. Hoehn, Tschertkow and Zipp blocked rabbits with ink and vaccinated them intracutaneously. Although there was no difference in the formation of the vaccinia pustula, in the blood of blocked animals the virus could be demonstrated on the fourth and fifth day after the vaccination while it was not found in the blood of the controls. Similar observations were reported by Walthard. In the guinea pig a generalization of the virus is difficult to obtain but after blockade and splenectomy the virus can be easily detected in the blood (Goldmann). The incubation is shortened, the reactive changes are less marked and subside quicker (see also Zurukzoglu and Joffe). The intracutaneous response to the vaccinia virus in the rabbit is entirely modified and even abolished when the virus is

mixed with India ink before injection or when it is injected into an area blocked with ink (Ledingham). Since infiltration of the skin with other material, e.g., streptococci, may also modify the reaction Ledingham concludes that it is the local stimulation of the histocytes which enhances the defense forces and leads to a quick destruction of the virus. Jungeblut discusses the possibility that the virus may pass quicker through the blocked area into the blood. On the other hand, Gildemeister and Heuer deny that the reticulo-endothelial system has anything to do with the generalization of the virus. Little has been done so far on the affinity of other invisible virus to the histocytes. Lépine and Schoen observed no effect of ink blockade on the susceptibility of the rabbit to the virus of epidemic encephalitis.

As an indication of the relation between latent infection and reticulo-endothelial system the appearance of microorganisms belonging to the bartonella and grahamella group in the peripheral blood of small splenectomized rodents might be mentioned (Mayer, Borchardt and Kikuth, Lauda, Lauda and Sorge, Cannon, Tagliaferro and Dragstedt, Nauck, Jaffé and Willis, Schwarz, and others). In rats the manifest bartonella infection causes a severe and often fatal anemia. Cannon and McClelland studied the effect of blockade on the bartonella infection of the rat and found that in splenectomized animals that had recovered from the anemia blockade caused a reappearance of the bartonellae in the blood. But they did not succeed in producing a bartonellosis by blocking normal rats though the animals became anemic.

The significance of the reticulo-endothelial system in silent infections has not yet been studied. An infection is called silent if it leads to immunity without causing any symptoms. It is different from latent infection in which the virus remains in the body (Nicolle, Doerr, Breindl).

RETICULO-ENDOTHELIUM AND GENERALIZED IMMUNITY. In actively immunized animals the response of the reticulo-endothelial system to the reinjected specific antigen is intensified. This intensification can be demonstrated under the microscope when bacteria are used as formed antigens. Domagk found a marked increase of the phagocytosis of various cocci by the reticulo-endothelial cells of the liver, spleen and lymph nodes of mice immunized against these cocci. Singer describes that pneumococci type III injected into the bone marrow of immune rabbits are quickly taken up and destroyed by the local reticulum cells and that blockade causes a loss of the immunity. The increase of the phagocytosis is, however, not restricted to the bone marrow, but is part of a generalized activation of the reticulo-endothelium. Similar

conditions exist in streptococci infections the immunity against which can be destroyed by injections of India ink (Bass).

Pneumococci type III brought into the abdominal cavity of mice immunized against them are rapidly phagocyted by macrophages. Newfeld and Meyer report that iron blockade and splenectomy prevent the active immunization of mice against this type of pneumococci and since in the serum of immune animals antibodies against pneumococci III cannot be demonstrated (Chickering, Cole and Moore, Cecil and Blake, Yoskioko, Tillet) Singer and Adler conclude that the immunity is due to a change in the reactivity of the histocytes. But also in infections with pneumococci type I and II which are associated with the formation of specific antibodies the activity of the reticulo-endothelial system seems to be the deciding factor because blocked immune animals though their serum may be rich in antibodies behave like normal controls (Singer and Adler). Neufeld and Meyer, however, maintain that humoral antibodies with phagocytosis-promoting properties (bácteriotropines) may have something to do with the antipneumococci immunity, an explanation which is supported by observations of Loewenthal and Micsch on tissue cultures of splenic macrophages infected with pneumococci; namely, cultures of macrophages from normal rabbits phagocyte avirulent pneumococci only in the presence of fresh normal serum, and virulent pneumococci only when immune serum has been added. Macrophages of immune rabbits take in both avirulent and virulent pneumococci without normal or immune serum being necessary for this process. If, however, the cultures are transferred to a new medium the macrophages lose their activity. Sufficient bacteriotropic substances were apparently present in the original culture into which they had been brought with the small amount of tissue fluid in the original explantate. Studies of Bloom on the phagocytosis of pigeon erythrocytes in cultures of rabbit's lung speak in the same sense. In cultures of the lung of normal rabbits the foreign erythrocytes group themselves about, and adhere to the local histocytes but are seldom phagocyted. In the lung cultures of immunized rabbits there is an intensive erythrophagocytosis. The same result can be obtained with the cultures of normal lungs when diluted fresh or inactivated immune serum is added. Because of the failure of demonstrating free complement in various tissue cultures, Bloom believes that phagocytosis takes place in the presence of heat resistant immune bodies. For a discussion of the questioned identity of the normal and immune opsonines (bacteriotropines) I refer to the recent publications of Neufeld and Wherry. The mechanism of the

opsonic and bacteriotropic action has been cleared by the interesting studies of Newfeld and Strumia, Mendel, Mendle, Lucké and McCutcheon.

While Bloom's study on tissue cultures indicates an increase of the erythrophagocytosis after immunization, in the living animal this mechanism is of much less importance. Only in young animals which are poor antibody producers immunization leads to an active phagocytosis of the foreign blood cells in the alveolar septa of the lung (Becker). In adult immunized animals extensive and rapid extracellular hemolysis is the response to the reinjection of the homologous erythrocytes (Levaditi, M'Gowan, Gerlach and Finkelday). This leads us to the discussion of the reticulo-endothelial cells as source of the antibodies.

The voluminous literature on the site of antibody formation has been recently reviewed by Howell, Sachs and Schlossberger and the reader may be referred to them for a detailed discussion of this problem. Since Pfeiffer and Marx's fundamental studies the interest of many investigators has been concentrated about the blood forming organs (spleen, liver, bone marrow, lymph nodes). In these organs antibodies can be demonstrated before they can be detected in the blood and in these organs their concentration first is higher than in the blood, to decrease later and become surpassed by that of the blood. The earlier work either did not distinguish between the different cellular elements of which the organs were composed or emphasized the importance of the lymphatic or myeloid tissue. The results of removing the spleen were not uniform because they were obscured by the quick compensation of the loss of the spleen by other organs (Russ and Kirschner). Other methods devised to secure a more generalized injury to the blood forming organs (injections of benzol and benzene or of radioactive substances, or exposure to roentgen rays or ultraviolet rays) are too crude and involve the other tissues too much to allow definite conclusions. The blockade of the reticulo-endothelial system was, therefore, welcomed as a new method to attack the problem. It is with the aid of this method that most of the recent work on the origin of the antibodies has been carried out but even with this method the most contradictory results have been obtained. The great affinity of formed antigens to the histocytes naturally points to these cells as the antibody producers and it seems very likely that under the influence of the storage of the antigen the cells liberate the specific reaction products, be it by using part of the antigen for building up the antibodies (Herzfeld and Klinger, Manwaring, and others), be it by the antigen acting as a specific meta-

bolic stimulant. It was expected that when the histocytes are blocked and unable to store the antigen antibody formation would not take place. Alone or in combination with splenectomy the effect of the blockade on the production of the coagulating, lytic, antitoxic, complement fixing and phagocytosis promoting antibodies has been followed by many investigators. The differences in the results obtained are readily understood if one keeps in mind the difficulty of an even temporary functional depression of the histocytes. One must remember that we are seeking the source of substances the nature of which is so uncertain. It has not even been decided how many different antibodies exist, whether the different immune reactions are different expressions of the same reacting substance or whether each of these reactions has its own specific substratum. The unitarian hypothesis revived and stressed by Zinsser has the advantage of simplying matters but so far has not been able to meet with all the objections (Wells).

Of the authors who succeeded in suppressing antibody formation by blocking the reticulo-endothelial system (with or without removing the spleen) I mention Bieling and Isaac (hemolysin), Siegmund (hemolysin, hemagglutinin), Gay and Clark (hemolysin, bacteriolysin), Jelin (agglutinin, bacteriolysin, complement fixing antibodies), Stewart and Parker (precipitin), Cannon, Baer, Sullivan and Webster (hemolysin), Vannucci (agglutinin), Kobagashi and Shiwotsu (agglutinin), Newfeld and Meyer (bacteriolysin), Isaacs (hemolysin and precipitin). Using the same methods negative results or a stimulating influence were recorded by Benassi, Bruyhnoghe and Collon, Collon, Fraenkel and Gruenberg, Howell and Tower, Howell and Beverly, Leontjeff-Warshatow, Rosenthal and associates, Ross, Standenath, Weiss and Kunze, Weiss and Sümeji, and others. The effect of blockade on the formation of diphtheria antitoxin was studied by Jungeblut and Berlot who found that India ink injections delayed the appearance of the antitoxin but did not prevent it. Collon, Fraser and Levinson and Golubewa saw no depression of the antitoxin production after blockade.

The effect of the blockade on the complement content of the serum has been investigated by several authors, notably Jungeblut, Siegmund, Landsberger, Stewart and Parker. According to Siegmund, Landsberger and Stewart and Parker there are no relations between the reticulo-endothelial system and the complement content of the serum. Jungeblut observed that there was a marked drop in the complement titer which started fifteen minutes after the blockading injection and reached its maximum in the third hour to return later to the norm or to

be slightly increased. The drop could not be due to an adsorption by the ink particles because the ink quickly disappears from the circulating blood. Merlini found that radiation in vivo or in vitro does not influence the complement.

Because of its unreliability the blocking method did not prove beyond doubt that the reticulo-endothelial cells are the source of the antibodies. There are, however, a number of other observations which support this conception. The morphological findings have already been referred to (see also Motahashi, Cary, Jones, and others). Young animals are poor antibody producers (Freund, Kligler and Olitzki, and others) and it has been shown by Becker that their reticulo-endothelial system was not yet fully developed. Grinding up organs with distilled water, Saxl and Duvall found especially bone marrow, spleen and lung rich in bactericidal substances. Valuable results have been secured from tissue cultures of the organs belonging to the reticulo-endothelial system (spleen, lymph-nodes, bone marrow, omentum). The first to demonstrate antibodies (hemolysins) in tissue cultures of lymph-nodes and bone marrow were Carrel and Ingebrigsten. This method has been later successfully used also by Przyode (agglutinin, precipitin), Schilf (bacteriolysin), Loewenthal and Micsch (agglutinin) and others, while Kuczynski, Tennenbaum and Werthemann and Bloom had negative results. Loewenthal and Micsch state that in older cultures, because of a differentiation of the histocytes into fibroblasts, the formation of antibodies ceases but Fischer was able to immunize cultures of fibroblasts against foreign proteins. Carrel and Ebeling describe the appearance of antibodies in cultures of leukocytes.

The increased output of antibodies that, according to the Danish school of serologists (Madsen and associates) follows the injection of small amounts of anorganic metal salts, in particular of manganese compounds, has been explained by Neufeld, Louros and Scheyer and Singer on the base of a stimulating effect of these salts upon the histocytes. Injected into guinea pigs infected with typhus, manganese chloride causes the formation of agglutinins to B. proteus X, 19 (Singer). Hogan's results with manganese injections were irregular.

As far as the immunity against protozoa is concerned, the humoral antibodies seem to be of less importance than they are in bacterial infections (Bieling). But in protozoal diseases, too, humoral antibodies have been demonstrated and their formation has been brought into relation with the reticulo-endothelial system. Thus, Kritschewski, Gruenbaum and Shapiro emphasize that the immunity against recurrens

spirochetes is essentially lytic in character and that phagocytosis by histocytes (Metschnikoff spoke of a phagocytosis of the spirochetes by microphages) is of lesser importance and occurs only in the terminal stages of the disease. In blocked and splenectomized animals there is an enormous increase of the spirochetes which in the blood vessels of the lung and bone marrow form dense coils. In unblocked animals the disintegration of the spirochetes can be observed under the microscope. In the central nervous system the spirochetes are not destroyed while in the choroid plexus they are. Taliaferro found that the resistance of rats against trypanosoma Lewisi is due to an antibody which inhibits the mitotic division of the nuclei of the trypanosomes. This inhibitory "reaction product" can be transferred to normal rats. According to Regendanz and Kikuth and Taliaferro, Cannon and Goodloe the spleen seems to be an important factor in the production of the reaction product since in splenectomized rats there is an unchecked increase of the trypanosomes. In this connection it may be remembered that blockade paralyzes the trypanozidal action of normal human serum in mice infected with trypanosomes (Rosenthal and Spitzer).

Summarizing the recent work on the site of antibody formation one has to admit that although many observations favor the theory that the antibodies are the product of the reticulo-endothelial cells the final proof is still lacking. The question will arise as to the importance of the humoral antibodies in the actively acquired immunity. Apart from the antitoxins and bacteriotropins the demonstration of antibodies in the infected organism was merely of diagnostic interest. There are no constant relations between the content of the blood as far as immune substances are concerned and the immunity of the individual and even in diphtheria, immunity may be present without the antitoxin being demonstrable in the blood. There are striking discrepancies between the appearance in the blood of immune bodies and the crisis in acute infectious diseases, especially in lobar pneumonia. Schilling states that in relapsing fever immune substances are not demonstrable before the onset of the crisis. In certain infections antibodies are formed against microorganisms which have nothing to do with the particular infection (typhus fever-B. proteus X 19 agglutinins, Weil Felix; tularemia-B. abortus and B. melitensis agglutinins; undulant fever-B. tularense agglutinins, Francis and Evans). Phagocytic immunity is independent of bacteriolysins and antitoxins (Newfeld) and the intracellular destruction of bacteria or foreign erythrocytes is much more thorough than is the destruction by the lytic substances of the serum (Newfeld). It

seems, therefore, that the defense mechanism of the body against infections is chiefly cellular in nature.

The rôle of the reticulo-endothelial system in passive immunity is still a matter of discussion and requires further studies. Neufeld and Meyer and Meyer saw no effect of blockade and splenectomy on the protecting action of antipneumococci serum and considered, therefore, passive immunity a humoral process. Jungeblut, on the other hand, noted that blockade interfered with the action of the antipneumococci serum. Tetanus toxin antitoxin mixtures are toxic or non-toxic to the same degree in blocked and non-blocked animals. When the antitoxin is given first and the toxin injected from twenty-four to seventy-two hours later the blocked animals are much less protected. Jungeblut discusses two possibilities, namely, an inhibition or retardation of the resorption of the antitoxin by the tissue cells or a hastening of the excretion of the antitoxin.

RETICULO-ENDOTHELIAL SYSTEM AND LOCAL IMMUNITY. According to Gay, local immunity rests on the demonstration that a given area of the body may be protected by the topical application of a given antigen without involving a more generalized immunity. There is an increasing evidence that such a localized, actively acquired form of immunity exists though it may be sooner or later followed by an immunity of the entire body. Besredka goes so far as to assume that general immunity is due to the local immunity of the sensitive tissue. Thus in anthrax, immunity is established when the skin, the chief port of entrance of the infection, is made impermeable to the anthrax bacilli with the aid of a cell desensitizing substance liberated from the breaking down of the specific microorganisms (antivirus). Outside of France Besredka's ingenious theory and its therapeutic deductions have so far found but little confirmation (Gay, Schlossberger).

As in the case of general immunity, local immunity may be the result of an increased cellular defense mechanism or of a local formation of antibodies. Gay stresses the importance of the cellular reaction attributing little if any significance to the production of humoral antibodies. He points out that among the different cells there is one type, which as far as the local increase of resistance is concerned, by far surpasses the other elements, namely, the clasmatocyte (histocyte, macrophage). Immunization either may make this particular type of cell more resistant or induce the acquisition of new properties or cause a transient local accumulation of these cells. The skin susceptibility to anthrax bacilli and other microorganisms then is due to a relative ab-

sence or slow mobilization of the protective cells which allow the bacilli to adapt themselves to the local conditions and to become invasive.

By injecting into the pleural cavity of rabbits suspensions of aleuronate or a gum arabic broth solution, Gay and his associates induced the formation of an exudate, the cellular composition of which was found to depend upon the lapse of time after the injection. The protective properties of this exudate were tested by a subsequent injection of highly virulent streptococci. After twenty-four hours the exudate is chiefly composed of polymorphonuclear leukocytes and does not afford any protection against streptococci even not in the presence of an anti-streptococcus immune serum. Later the exudate becomes rich in clasmatocytes and the parietal pleura is covered by a thick layer of granulation tissue containing a great many of these cells. When into this pleural cavity streptococci are injected they are quickly phagocyted by the clasmatocytes of the exudate and granulation tissue and the animal survives. After collection of the macrophages in one area they may move to another area, e.g., to the other pleural cavity and here exert the same protecting action (Gay, Clark and Linton, Linton). Nakahara induced a macrophagic reaction in the peritoneal cavity of mice by injecting olive oil and by means of this reaction protected the animals against intraperitoneal injections of staphylococci, pneumococci and B. coli. Stuppy, Cannon and Falk observed that the local immunization of the lung following a vaccination of the upper respiratory tract was associated with an accumulation of histocytes. Using the peritoneal cavity as test object, Opie found that the leukocytic exudate of the early stage temporarily protects the animal against an invasion of the blood stream by the streptococci but that the recovery from the infection depends upon the establishment of a later stage of the inflammatory exudate. He considers the possibility that the large amount of fluid of the early exudate may favor the multiplication of the cocci. Gay and Clark, however, were able to show that withdrawing of the leukocytic pleural exudate did not help to increase the resistance, not even when the removed fluid was replaced by immune serum. On the other hand, the same serum intensified the protective action of the exudate composed of macrophages.

The importance of phagocytosis promoting substances of the serum for the protective efficiency of the macrophages in the pleural exudate becomes evident when the streptococci are replaced by pneumococci type I. By merely producing an exudate and granulation tissue rich in macrophages Clark was not able to protect rabbits against an intra-

pleural injection of these cocci. In order to save the rabbits mixing the pneumococci with an homologous immune serum was necessary. Also Tudoranu obtained protection against pneumococci (type III) injected into prepared pleural cavities of the rabbit only when immune serum was given.

The local formation of antibodies is still a matter of discussion. If the histocytes produce the antibodies a local formation of these substances would seem to be possible since histocytes are ubiquitous. It is, however, the quick resorption of the antigen from the place of injection which shortens the time interval between local accumulation and general distribution of the antibodies so much that a separation of the two phenomena is exceedingly difficult, unless the antigen is administered to a region from which its resorption is less rapid, as for instance to the eye (Roemer, Paschen, von Dungern, Leber, and others; see, however, Hektoen). Th. Smith, Orcutt and Little maintain that injection of abortus bacilli into the udder of the cow causes an agglutinin content of the milk which is much higher than that in natural infections.

Wassermann and Citron state that the exudate at the point of injection of the antigen contains more antibodies than does the blood which, however, may be due to a local concentration of the antibodies of the blood. Schamburov among others believed that he had found evidences of a local formation of antibodies in the subarachnoidal spaces, but Freund emphasized the fact that antibodies of the blood may enter the subarachnoidal fluid even when the meninges are not inflamed. Portis removed the greater omentum of rabbits and found that after intraperitoneal injections of sheep or chicken erythrocytes or of sheep serum the antibody content of the peritoneal fluid was only one-fourth of that of the controls. Dogs and guinea pigs did not show these differences and Portis believes that it is the large number of histocytes in the milk spots of the rabbit's omentum which may account for these findings. But the omentum of the dog and guinea pig also contain many histocytes though in the dog they do not accumulate in grossly visible spots.

The effect of local blockade on a subsequent cutaneous infection was studied by Kuschnarjew who observed that the blockade prevented the local reaction but favored the entrance of the bacteria into the blood stream. Local blockade of the skin protects against an injection of diphtheria and tetanus toxin to a certain extent (Kuschnarjew, Ledingham).

Reticulo-endothelium and hypersensitiveness. Hypersensitiveness to antigenic substances has been repeatedly linked with the reticulo-endothelial system. Mautner and Luzzato state that removal of the spleen immediately before or after sensitisation prevents or prolongs the anaphylactic shock. The effect of the blockade upon the formation of the anaphylactic reaction body has been studied by Musante, Neufeld, H. Meyer, Isaacs, Jungeblut and Berlot, Simitch and Haendel and Malet and it seems from their findings that the development of the anaphylactic condition is not markedly influenced by this procedure. More striking results are obtained when the blockade, alone or combined with splenectomy, is done during the interval between sensitisation and reinjection of the antigen (Moldovan and Zolog, H. Meyer, Siegmund, Petersen, Jaffé, Levinson and Hughes, Landsberger, Jungeblut and Berlot, Haendel and Malet, Friede and Ebert, Schittenhelm and Erhardt, Fujoka). Using the strain of diphtheroid bacilli which invades all the endothelial cells of the mouse, Elmer and Hammerschmidt were able to suppress the shock in the mouse. Simitch and Isaacs, however, saw no shock preventing action of India ink, saccharated iron oxid or trypan blue given shortly before the reinjection of the antigen. Among the authors who succeeded in interfering with the shock, differences were encountered which were due to individual variations of the animals and to differences between the species which had been used. The most suitable animal appears to be the guinea pig (H. Meyer, Schittenhelm and Erhardt, Moldovan and Zolog, Haendel and Malet, Jungeblut and Berlot) and positive results also were obtained with mice (H. Meyer) and frogs (Friede and Ebert) while discrepancies were found when dogs or rabbits were tested (Musante, Jungeblut and Berlot, Schittenhelm and Erhardt, Petersen, Jaffé, Levinson and Hughes).

Landsberger tested the functional activity of the reticulo-endothelial system during the anaphylactic shock by determining the disappearance time of congo red from the blood stream and obtained irregular results, the function either being increased or depressed or not affected at all. As I have shown on a previous occasion, congo red is not suitable for testing the function of the reticulo-endothelium.

Splenectomy and blockade do not completely eliminate the hypersensitiveness but merely decrease it as shown by a prolongation of the shock or by the fact that larger doses of the antigen are necessary to call forth the shock. According to the present day conception, the anaphylactic shock is chiefly the outcome of intracellular processes which are, however, associated with certain changes in the blood (Wells).

Anchored to the cell, the anaphylactic antibody reacts with the homologous antigen bringing about the sudden, in their nature still unknown, changes culminating in the shock. There is evidence that the intracellular antigen-antibody reaction is not restricted to the reticulo-endothelial cells although this part of the reaction may be a very important one, but that the range of cells involved is much wider, including not only all the endothelial cells (Doerr, Domaghk) but also other structures, especially the smooth muscle fibers. It can, therefore, not be expected that blockade completely eliminates the hypersensitiveness.

There are some observations which do not fit into the explanation just advanced. If the reticulo-endothelium is so important for the anaphylactic shock, why is it then that cultures of spleen and lymph nodes of guinea pigs sensitized to horse serum are not affected when horse serum is added to the cultures but grow as normal cultures do (K. Meyer and Loewenthal), whereas the smooth muscle fibers of the sensitized animal contract when submerged into solutions containing the antigen? A new theory trying to explain the interference of the blockade with the shock has been recently advanced by Moldovan. Moldovan and his associates observed that the anti-anaphylactic state induced by injections of India ink can be transferred to other sensitized guinea pigs. His conclusion, therefore, is that the blockade leads to the excretion of an anti-anaphylactic principle which he was able to isolate from the blood. This principle called "reticulin M (Metschnikoff)" is a product of the reticulo-endothelial cells which excrete it under the stimulating influence of the blockade and which is supposed to inhibit the contraction of the smooth muscle fibers of the bronchi.

Of other observations which may be of interest in this connection, I quote the prevention of the anaphylactic shock of the guinea pig by a preceding injection of neoarsphenamin (Jungeblut) and the failure to produce an anaphylactic shock in guinea pigs infected with tuberculosis (Pagel and Garcia Frias).

H. Meyer and Fujoka state that blockade also prevents passive sensitization. Jungeblut and Berlot were not able to confirm this statement but observed that neoarsphenamin also protects against passive anaphylaxis.

Concerning the reversed anaphylactic shock, the investigations of Forssman and Skoog and Jungeblut and Newman dealing with the effect of the blockade on the action of the heterophilic antisera (Forssman sera) may be mentioned. Forssman and Skoog did not see any influence of India ink injections on the toxicity of the heterophilic sera while

Jungeblut and Newman gained the impression that ink and trypan blue prolonged the time of survival of the guinea pigs. The protecting action of the trypan blue was very striking when the heterophilic serum (anti-sheep erythrocyte and anti-guinea pig kidney cell serum of rabbits) was injected into a region of the skin which had been previously blocked by trypan blue. India ink did not protect and the animals prepared with it developed just as severe a local reaction with necrosis as did the untreated controls.

The local toxic action of the heterophilic sera leads to a discussion of the relations between Arthus phenomenon and blockade. Large amounts of ink given intravenously before and after sensitization do not influence the Arthus' phenomenon. Using trypan blue, half of the animals (rabbits) are protected (Klinge). Intracutaneous injections of trypan blue are very effective in preventing the hyperergic reaction provided the serum is reinjected into the vitally stained area before the granular storage of the dye is completed. As long as the active mesenchymal cells are irritated by the dye, they do not participate in the formation of the anaphylactic toxin (Klinge).

Freund describes that splenectomy combined with iron or trypan blue blockade prevents the development of the tuberculin hypersensitiveness of tuberculous guinea pigs. After the skin allergy is once established, the blockade has no effect. Freund believes that the reticulo-endothelial system is connected with the formation of the tuberculin hypersensitiveness which is analogous to the antibody formation. It has also been shown that young guinea pigs react very slightly to intracutaneous tuberculin injections or do not react at all (Freund, Valtis). The fatal tuberculin shock too can be prevented by injections of manganese chloride (Walbum) or India ink (Jungeblut). In tuberculous rabbits the allergic reactions are increased by cholesterol and metal storage and decreased by dye storage (Minami).

Seiffert's and Pagel's and Garcia Frias' experiences with blockade and tuberculin hypersensitiveness were different. Seiffert observed that blockade increased the sensitiveness of the skin to tuberculin and Pagel and Garcia Frias saw no effect on the tuberculin shock nor on the local skin reaction. Koch's fundamental experiment (reinjection of living tubercle bacilli), however, was distinctly influenced in the sense of depression by local ink storage. The same experience was made by Paraf. The depression of the local hypersensitiveness to tubercle bacilli by ink injections is surprising since this reaction is essentially leukocytic (Jaffé and Loewenstein).

Other hyperergic skin reactions have so far not been studied from the standpoint of the reticulo-endothelial system and it would be especially interesting to find out whether local blockade interferes with the Schwartzman reaction. In unpublished experiments I have found that local trypan blue blockade does not prevent the skin reaction produced in guinea pigs infected with Coccidioides immitis by injections of killed suspensions of this fungus or filtrates of broth cultures.

THE RELATIONS BETWEEN RETICULO-ENDOTHELIAL SYSTEM AND CHEMOTHERAPY. From the early days of chemotherapy the disproportion between the antiparasitic action of the drug in vitro and that in vivo has attracted much attention. Amounts of the drug too small to kill the microorganisms in the test tube are highly efficient when injected into infected animals. Ehrlich believed that inside the body the salvarsan was oxidized into a potent form. Thus, for the therapeutic effect of a chemical compound, the body of the host appeared to be just as important as the compound itself. Chemical studies on the distribution of salvarsan revealed that it soon disappeared from the blood to be stored in the liver and the spleen. Since storage of foreign substances is one of the outstanding functions of the reticulo-endothelial system, Schlossberger suggested that these cells also might be engaged in the deposition of the arsenobenzol. Many years ago Giemsa explained the action of quinine in malaria by a primary storage of the quinine in the endothelium of the liver and the spleen from where it would be transferred to the plasmodia. As to the sanocrysin it is known that it displays its greatest activity in the presence of irritated tuberculous granulation tissue (Madsen and Moersch). Tuberculous granulation issue is chiefly composed of histocytes. Morphological evidences of a stimulating action of chemotherapeutic compounds upon the reticulo-endothelial system have been described by Goldzieher and Peck.

With the aid of blockade and splenectomy, important functional relations between chemotherapy and reticulo-endothelial system could be demonstrated. This new function of the reticulo-endothelium was first described by Kritschewski and Meersohn and shortly afterwards and independently by Feldt and Schott and Jungeblut. In the experimental infection of the mouse with recurrens spirochetes or trypanosomes, depression of the function of the reticulo-endothelium interferes greatly with the chemotherapeutic efficacy of salvarsan (Kritschewski and Meersohn, Feldt and Schott), neo-silver salvarsan (Jungeblut), Bayer 205 (Feldt and Schott, Jungeblut), solganol, trypaflavin, anti-

mosan (Feldt and Schott). At first Kritschewski believed in a combination of the etiotropic action of the drug with the natural defense mechanism of the body. Jungeblut and Jimenez de Asua and Kuhn assumed that the reticulo-endothelial cells transformed the drug into its active form. Feldt and Uhlenhut spoke of a stimulating influence of the chemotherapeutic substance upon the functional activity of the active mesenchyma. Even as potent a drug as Bayer 205 needs the cooperation of the body of the host in order to exert its full therapeutic effect (Reiner and Leonhard).

Kritschewski later changed his opinion, emphasizing that it is the quick initial storage and gradual subsequent release of the drug by the reticulo-endothelium which secures the best effect. This mechanism prevents the excretion of the drug before it has had a chance to attack the microorganisms. Jungeblut accepts this new conception of Kritschewski to which he lends support by demonstrating that in the blocked animal the arsenicals are removed from the blood much slower than in normal animals and that the blocked liver contains only half the amount of arsenic which is found in the liver of the controls. The blockade shifts, therefore, the distribution of the arsenobenzol in favor of the blood stream. Reiner, Leonhard and Chao, working with sodium atoxylate, state that blockade does not diminish the arsenic content of the liver but that of the spleen.

The storage in the body of arsenicals, especially of the less diffiusible trivalent compounds, had been previously described by Voegtlin and Tompson and Voegtlin, Dyer and Miller pointed out that this storage favored the therapeutic action. With the aid of histochemical reactions, the histocytes were found to be responsible for the storage of the arsenobenzols (Von Jancso, Jimenez de Asua and Kuhn). The absolute storage was greatest in the liver while the spleen showed the largest relative storage (Von Jancso). In addition there were arsenobenzol emboli in the capillaries of the lung (Von Jancso) and an impregnation of the perivascular connective tissue (Jimenez de Asua).

Not all the arsenicals are stored to the same extent in the reticulo-endothelium (Von Jancso). The most intensive storage is obtained with silver salvarsan while neosalvarsan and sulfoxyl salvarsan are stored very little and arsalyt not at all. It is of great interest that the intensity of the storage does not correspond to the efficacy of the drug, at least in the experimental recurrens infection of the mouse (Von Jancso), solfoxyl salvarsan and arsalyt curing promptly the infected animal. In experimental trypanosomiasis there is, however, a distinct

parallelism between storage and therapeutic action, silver salvarsan being more effective than arsalyt. With regard to the prophylactic action in the recurrens infection of the mouse, Von Jancso has found that the silver salvarsan which is so promptly deposited in the reticulo-endothelial cells, after forty-eight hours loses its protecting power. The best results are obtained with the aid of sulfoxyl salvarsan which is stored very little and remains in the blood for seventy-two hours. Though the reticulo-endothelial cells are filled with arsenobenzol granules, the recurrens infection takes as it does in untreated controls (Von Jancso). Kritschewski explains Jancso's prophylactic results by the high resistance of the spirochaeta Duttoni against arsenic. Using the more sensitive trypanosomes there was also in the prophylactic experiments a perfect correspondence between storage and protecting action.

Splenectomy increases the toxicity of sodium cyanide, caffein, and morphin and of the etiotropic drugs (trypan red, tryparosan, atoxyl, trypaflavin) (Kritschewski). Kikuth and Regendanz found that splenectomy diminishes the therapeutic effect of Germanin. Kritschewski, who confirmed Kikuth's and Regendanz's observation, was able to compensate to a certain extent for the loss of the spleen by giving the drug in 6 per cent agar instead of in saline solution. The agar keeps back the drug and discharges it gradually into the blood as the spleen does in the normal animal. As with the other functions of the spleen so, too, the storage of the drug is soon taken over by the other parts of the reticulo-endothelial system when the spleen is removed (Kolpikow, Rubinstein and Golubewa).

The theory of Kritschewski concerning the significance of the storage of the chemotherapeutic substances in the reticulo-endothelium presupposes that the cells take up and give off these substances without changing their chemical character. This holds true for the benzidine dyes (trypan red, trypan blue) and for the Germanin which is closely related to them (Schlossberger). The arsenobenzols are retained by the histocytes over a long period of time. Von Jancso says that it takes twenty days before the arsenobenzol granules disappear from the stellate cells of the liver. During this long stay, however, the arsenicals gradually break down to inorganic compounds which are excreted with the bile (Schlossberger). By this breaking down the arsenicals lose most of their antiparasitic action. On the other hand the blocked mouse has more salvarsan in the blood than the normal control and is less protected against trypanosomes than the latter, the histocytes of which are filled with salvarsan granules.

But even in the chemotherapeutic studies the result of the blockade is not uniform. Reiner, Leonhard and Chao, who had found a very active antiparasitic agent against trypanosomes in the blood of animals injected with arsenicals, blocked rats and rabbits with India ink. Twenty hours later the animals received an injection of sodium atoxylate and were bled after twenty-four hours. The blood of the blocked animals was found to possess the same potency as did the blood of the controls. Sometimes the potency was even increased and the authors conclude, therefore, that the blockade does not inhibit the formation of the antiparasitic substance but may even enhance it.

The question becomes still more complicated by Seiffert's experiments on the effect of blockade and splenectomy on the therapeutic action of bismuth yatren in the experimental infection of the guinea pig with the spirochaeta ictero-hemorrhagica. Seiffert found that the blocked and splenectomized animals were cured by the same dosage of yatren as were the controls. Kritschewski thinks that yatren does not kill the spirochetes of infectious jaundice but in a yet unknown manner prevents their pathogenicity.

The difficulty of correlating the different and often contradictory results of the experiments on reticulo-endothelium and chemotherapeutic action induces Schlossberger to reject the explanations given by the other authors. Blockade and splenectomy do not act only upon the reticulo-endothelium but stimulate and accelerate the entire metabolism. According to Schlossberger, it is this acceleration of the metabolism which causes a more rapid breaking down of the chemotherapeutic drugs and hence diminishes their efficiency. But also this explanation cannot satisfy fully.

Although many important questions remain to be answered, the experiments have clearly shown that first, there are differences in the way of combating infections and protecting against infections between the different chemotherapeutic compounds, and second, that the mechanism of action of a given drug seems to depend upon the type of infecting microorganisms (spirochete, trypanosome).

RETICULO-ENDOTHELIAL SYSTEM AND TUMOR RESISTANCE AND IMMUNITY. The important rôle of the reticulo-endothelial system in microbic infections and immunity suggests that this system may also be concerned in the susceptibility and resistance to malignant tumors. The infrequency of gross metastases of carcinomas to the spleen has long been known and has often been brought in relation with the great number of histocytes in this organ. Microscopic examination not

seldom reveals small colonies of tumor cells in the spleen, but the cells do not proliferate as they do in other organs. Lack of metastases in the spleen, however, cannot be used as an argument in favor of the significance of the reticulo-endothelial system in tumor immunity since other organs rich in these cells are the most common sites of secondary invasion (liver, lymph nodes, bone marrow). Apolant, Oser and Pribram and others found that removal of the spleen in mice and rats favors the take of transplantable tumors. Even those spontaneous tumors which cannot be transferred to other animals grow in splenectomized animals (Brüda). Dobrovolskaia-Zavadskaia and Samssorow did not experience any difference of the tumor growths after splenectomy and denied that the spleen had anything to do with the susceptibility of mice and rats to transplantable malignant tumors. Similar negative results were recorded by Rothenburg, Fibiger. These authors perhaps waited too long after splenectomy since it had been shown by Brüda that the loss of the splenic tissue soon was compensated by other parts of the reticulo-endothelial system. Splenic tissue added to cultures of tumor cells in vitro depresses the tumor growth (Murphy, Carra), while the cells grow particularly well in the plasma of splenectomized animals (Brüda).

In experiments on heterologous tumor transplantation to be discussed later, the depressing action of the spleen could also be demonstrated (Murphy and Sturm, Brüda). Fischera and Brancati report that tumor cells mixed before inoculation with splenic or thymic tissue take poorly in the new host. In similar experiments with splenic pulp, Kuroren could not confirm this observation. Braunstein, Biach and Weltmann and Oser and Pribram describe that injections of splenic pulp into tumor bearing rats and mice cause a distinct regression and sometimes even a complete disappearance of the tumor. Extracts of spleens of mice injected with the blood of tumor mice are particularly potent (Braunstein). Pitzman, however, found that splenic tissue did not yield immunity against transplantable tumors and Blumenthal succeeded in producing tumors in rats with the spleen of tumor rats. This later result is explained by Braunstein by the presence of single tumor cells in the spleen of the tumor animals.

Borghi emphasized that tarring of mice leads to a severe damage of the reticulo-endothelial system and also Domagk mentioned the injurious effect of tar painting upon the liver and spleen. Animals inoculated with transplantable tumors, in the early stages of tumor growth, show an hypertrophy and hyperplasia of the reticulo-endothelial cells to be

followed by atrophy after the tumor has fully developed (Borghi). Weak radiation makes mice more resistant to tumor grafting, intensive radiation makes them more susceptible. The effect of radiation is interpreted by Vorländer as due to a stimulation or depression of the histocytes, while Murphy refers it to an alteration of the lymphocytes.

Several investigators studied the effect of the blockade on tumor resistance and susceptibility. Rhoda Erdmann blocked mice with India ink and succeeded in producing tumors by injecting cell free filtrates of tumors or suspensions of tumor cells treated with chloroform. In normal animals no tumors could be obtained by this material. Blockade with dyes had no effect and Erdmann concludes from her experiments that the ink stimulates the reticulo-endothelial cells to give off a substance which is essential for tumor formation. While Haagen could confirm Erdmann's results, Büngeler was unable to produce tumors with the aid of cell free tumor filtrates. In some of the intensively blocked mice there was a take of chloroform injured tumor grafts. Büngeler does not believe in a stimulation of the histocytes and rather is inclined to assume a depression of these cells. Loewenthal and Wibeau injected into ink blocked rats Berkefeld filtrates of the highly infectious ascites fluid of rats with Flexner-Jobling tumors, but their results were negative.

Stimulated by Lehmann's and Tamann's experiments on successful homoioplastic skin grafts in blocked mice, Lignac and Kreuzwende dich vor dem Borne implanted a large celled mouse sarcoma into normal mice and into mice vitally stained with trypan blue. The staining caused a marked increase in susceptibility. The latent period was shortened, the tumors grew faster, formed more extensive metastases, reached a larger size and the animals died sooner. Reimplanted into normal mice, the virulence of the tumor was distinctly diminished. On the other hand, Munk observed a depressing action of the vital staining upon the growth of transplantable mouse tumors.

Highly diluted suspensions of tumor cells which, after intracutaneous inoculation, do not take in normal mice do so in blocked mice (Urbach and Schnitzler). Fischer Wasels mentions that intensive local blockade favors the growth of an implanted tumor. Brüda states that tumor cells show an excellent growth in plasma of blocked animals. While Kreuzwende dich vor dem Borne was unable to produce, by tar painting, tumors in mice injected with trypan blue, Rudolf Jaffé did not see any effect of ink blockade on experimental tar tumors.

Of particular interest are the experiments with heterologous tumor transplantation. Paul Ehrlich described that a transplantable tumor

inoculated into an animal of another species might grow for a short period of time. This initial growth soon was followed by regression and resorption. Numerous investigators confirmed Ehrlich's observations and only in embryo successful heterologous tumor growth was accomplished (Murphy). Shirai later reported that he could produce heterologous tumors when the tumor was grafted into the brain. Murphy and Sturm implanted pieces of mouse sarcoma into the frontal lobe of the brain of rats, guinea pigs and pigeons. The tumor grew with great rapidity but when it came in contact with the ventricles it was destroyed. Simultaneous inoculation of the tumor with an autograft of the spleen showed a marked inhibition of the tumor growth which was not the case when the autograft was substituted by a homograft (Murphy and Sturm). By grafting adult splenic tissue also embryos can be made resistant against heterologous tumors. Gheorghiu repeated Shiari's and Murphy's and Sturm's experiments and found that the growth of the mouse tumor in the brain of the rat is limited and that after a month the tumor becomes penetrated by phagocytic mononuclear cells. Similar results can be obtained by intratesticular inoculation.

It has been suggested that Shirai's and Murphy's and Sturm's successful intracerebral inoculation of heterologous tumors may be due to the fact that the central nervous system does not possess reticulo-endothelial cells (Brüda). Even if we assume that the Hortega glia does not have any protecting properties, the brain and spinal cord cannot be called free from histocytes since the blood vessels are surrounded by adventitial cells as they are in the other organs. These adventitial cells, no doubt, take an important part in the defense reaction of the central nervous system.

Roskin transplanted chicken sarcoma into blocked mice. Nine of twenty mice developed a tumor which reached its maximum after sixty days and then gradually disappeared. He also observed occasionally a growth of mouse carcinoma in blocked chickens and of human breast cancer in blocked mice. A large series of experiments on heterologous tumor transplantation in mice and rats was reported by Brüda. When the reticulo-endothelial system had been weakened by removal of the spleen, blockade or intensive radiation Brüda was able to obtain up to 90 per cent takes of mouse tumors in rats and vice versa. It was necessary that the inoculation was done soon after splenectomy because within from three to four weeks the rest of the reticulo-endothelium had taken over the functions of the spleen. Blocking these animals restored the susceptibility to heterologous tumors. The tumors were large, kept

on growing till the animal died, could be reimplanted into the original species and could also be transferred to splenectomized members of the new species; but in the latter instance, the positive results decreased to 45 per cent.

Removal of the spleen makes the plasma of rats a good culture medium for mouse sarcoma and carcinoma. Brüda, therefore, believes that in the normal organism the spleen excretes a substance directed against excessive pathologic growth. In order to prove this conception, Brüda and Pfeiffer studied the effect of parabiosis on the tumor growth in splenectomized animals. When a splenectomized rat is united with a normal rat the heterologous tumor graft does not take in the splenectomized animal. The splenectomized animal acquires from his normal companion the protective substance and since it is connected only through the blood, this substance must be circulating in the blood. This substance apparently excreted by the spleen is called by Brüda mesenchymin in analogon to the reticulin which Moldovan isolated in his experiments on anaphylaxis in blocked animals. Brüda and Pfeiffer extended the parabiosis experiments also to tumor bearing animals. Here the symbiosis had only a temporary effect and, after an initial inhibition, the tumors grew as they did before the parabiosis was performed. The mesenchymin, therefore, possesses protecting but no curative properties.

Roskin's and especially Brüda's experiments are still awaiting confirmation. Sternberg could not transplant chicken or human tumors into mice blocked by trypan blue, India ink, or saccharated iron. By blocking, Psaromita was unable to render mice susceptible to chicken sarcoma or human breast carcinoma. Tokiyasu observed only a better initial growth of heterologous tumors, but later the tumors disappeared whether the animals were blocked or not.

Zacherl tried to apply the results of the experimental studies on tumor immunity to the treatment of human cases of carcinoma. Using Kaufmann's method of the qualitative cellular picture of the content of cantharidin blisters as a criterion, he found a distinct decrease of the functional activity of the reticulo-endothelial system in patients with carcinoma. Intensive deep x-ray treatment caused a further drop of the lympho-histocytic elements of the blister content. When, however, repeated small doses of rays were given, there was a distinct rise of the mononuclear cells. This stimulation also could be accomplished by radiating the spleen or injecting splenic extracts prepared after the method of Brüda. Similar results were obtained with the aid of ultra

violet rays. The carcinolytic properties of the serum (Freund-Kaminer reaction) showed identical variations, namely, a drop after a single large roentgen dose and an increase to norm or even above after repeated small doses. Zacherl, therefore, states that the advantage of the intensive local destruction of the tumor tissue by a single large dose is more than compensated by its injurious effect upon the reticulo-endothelial system, whereas repeated small doses, though locally less effective, help the body combatting the tumor by stimulating the reticulo-endothelium.

The question arises as to the mechanism by which the reticulo-endothelial cells influence tumor growth. Are resistance and immunity cellular or humoral? In the latter instance, are the antiblastomatous substances a product of the histocytes? Uhlenhut believes that as far as tumors in lower animals are concerned resistance and immunity are cellular in nature. The graft which does not take is surrounded by histocytes and granulation tissue. Murphy and Nakahara attributed much significance in tumor immunity to a lymphoid cell proliferation. In human cases of carcinoma, an intensive local histocytic reaction does not give a better prognosis than does a plasmocellular-lymphocytic or granulocytic wall. (Own observation.) The reticulo-endothelial constituents of the spleen cannot account for its relative immunity against malignant tumors and its antiblastomatous properties because the lymph nodes and the liver too are very rich in these cells and are the most common site of metastases. One would have to assume that the histocytes of the spleen are endowed with properties which the other parts of the reticulo-endothelial system do not possess. There is no proof that such differences between the histocytes of the spleen and those of the other organs exist. Lambert and Hanes observed that tumor cells grow just as well in the plasma of naturally resistant or artificially immunized animals as they do in the plasma of normal or tumor bearing animals. Their conclusion is that there is no specific cytolytic substance in the body fluids of the animals immune against transplantable tumors. Interesting contributions to the question of tumor immunity were published by Lumsden. According to this author, secretion products of leukocytes seem to be of importance for the acquired immunity against homologous tumors. In order to destroy tumor cells in vitro, the serum of the immune animal has to be activated by substances derived from the leukocytes. No such substances are necessary when the animal is immunized against heterologous tumor cells.

This brief discussion may suffice to illustrate the intricacy of the ques-

tion of tumor immunity. Much work still remains to be done and more criticism is necessary when it comes to consider the part played by the reticulo-endothelial system. The increase of the carcinolytic properties of the serum which follows radiation does not warrant the explanation that this phenomenon is due to a stimulation of the histocytes. X-rays do not act only upon the histocytes but also on many other cells, blood cells as well as parenchymatous cells. In particular Brüda's successful heterologous tumor transplantation needs confirmation. It has been repeatedly shown that the tumor cell is species specific (L. Loeb, Jensen, Roussy a.o.). Dungern who succeeded in transplanting a sarcoma of a hare into rabbits found with the aid of the precipitin reaction that the cells remained hare cells. In heterologous tumor grafts the tumor cells retain the morphological appearance of the original tumor though their arrangement and the amount of stroma may differ (Lumsden). Roskin, however, describes a change in the histological structure of the successfully transplanted heterologous tumor. If heterologous tumor transplantation is really possible,—not only a temporary growth but an unlimited growth until the animal dies from it,—one would have to investigate with the aid of immunological reactions (anaphylactic shock and precipitin reaction), whether the tumor cells remain cells of the animal species from which they have developed or whether they change their specificity and become cells of the new host. Lumsden considers the possibility that the tumor cells of the different animals may have something in common, a common antigenic substance.

CONCLUSION

Although opinions may differ whether the separation of the reticulo-endothelial system as a functional unit is justified, the reinvestigation of the mechanism of resistance, infection and immunity as the outcome of this conception marks a distinct step forward in the understanding of the relations between host and microörganisms. The study of the microörganisms inside the body has clearly demonstrated the paramount importance of the cellular defense. Among the cells engaged in the defense reactions the histocytes, fixed and mobilized, play a predominant rôle. They not only engulf and destroy the microörganisms but also seem to be the source of those substances, the appearance in the blood of which indicates the response of the body to the infection. When the microörganisms are able to escape or to resist the histocytes, their increase in the blood and tissues is unchecked and death results. One should, however, avoid making the mistake of the humoral period of

immunology which tried to explain resistance and immunity chiefly by the action of the free antibodies, by stressing one factor too much and neglecting other possibilities. Thus, the leukocytic mobilization and phagocytosis have to be taken into consideration though the leukocytes are apparently less potent phagocytes than are the histocytes, to which they are genetically related. The significance of the leukocytes is not only illustrated by the observations on animals made aleukocytic, but also by experience in human pathology. Anergic or dysergic conditions of the granulopoiesis causes a marked decrease of the resistance. I mention the severe, rapidly progressing necrotic lesions of the mucous membranes in cases of agranulocytosis, aplastic anemia and acute leukemia in which the reticulo-endothelial system apparently is not injured.

One question has so far not been brought up, namely whether the cellular defense reactions, in particular the action of the histocytes, is influenced and controlled by the nervous system. There is still disagreement as to the modification of the local inflammation by denervation. The phagocytosis in surviving, perfused organs (Manwaring), the immune phagocytosis and antibody formation in tissue cultures, suggest the independence of these processes of the nervous system. Inside the body with all the organs in intimate relations to each other, the conditions are perhaps different as shown by the experiments of Bogendoerfer who succeeded in preventing agglutinin formation by severing the cervical cord of dogs shortly before the injection of typhoid vaccine. When, however, this operation is done from one-half to twenty-four hours before the injection of the antigen, the antibody formation is not interfered with. Severing the cervical cord separates the sympathetic centers of the cord from the higher centers. It seems, therefore, that the sympathetic nerves are associated with antibody formation. Paralyzing the sympathicus by gynergen has the same effect as cutting the cervical cord (Bogendoerfer). It is interesting to note that this operation does not affect the vital staining with trypan blue (Letterer and Bogendoerfer). Metalnikow found that in a type of caterpillar which readily forms antibody to cholera vibrios, destruction of the third dorsal ganglion prevents immunity. Pawlow's conditional reflexes increase the output of antibodies (Metalnikow, Vigodchikoff and Barykine, Podkopaeff Saotchian, Nicolan and Antinescu) and according to Metalnikow the immunity is controlled by nervous centers as are other physiological phenomena.

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SECRETIN

EUGENE U. STILL

Department of Physiology, University of Chicago

It is impossible to treat the subject of this review without allusion to other hormones and physiologically active substances found in the mucosa of the upper intestinal tract. These substances, in the main, have been discovered as the result of investigations upon the physiology and chemistry of secretin.

In 1893 Bekker and in 1894 Dolinski (40), students of Pavlov, made the observation that the application of acids to the mucosa of the duodenum provoked a flow of pancreatic juice. This observation caused many investigators to interest themselves in the mechanism by which the acid caused the pancreas to secrete. Popielski (164) and Wertheimer and LePage (198) believed the response was due to a peripheral reflex and finding that the response diminished with descent down the intestine concluded that the reflex was a local one, probably involving only the duodenum and pancreas.

Bayliss and Starling were attracted by these observations, especially that the acid-reflex diminished at lower levels of the intestine and in 1902 (10) proposed a hormone mechanism for the external secretion of the pancreas. This theory was founded upon three observations: first, the application of acid to a denervated loop of jejunum provoked a secretion from the pancreas; second, the intravenous injection of an acid extract of the mucosa of the upper intestine caused the pancreas to secrete, and at the same time caused a marked vasodilatation; and third, similarly prepared extracts from other tissues were without effect upon the pancreas, but caused vasodilatation. Due to the fact that extracts prepared with water or salt solution contained the dilatin substances but had no effect upon the pancreas, they proposed the existence of a substance, prosecretin, which was activated by acids. They believed the active substance to be a specific one for the pancreas and suggested the name "secretin."

Bayliss and Starling considered their experiment on the denervated loop of intestine crucial. In 1902 Wertheimer (197) confirmed their

observation but questioned the completeness of the denervation as did Luckhardt, 1922 (119). Popielski, 1902–1907, (160–163) also confirmed the observation of Bayliss and Starling but claimed that after ligature of the pylorus, the application of acid to the denervated loop no longer led to a secretion of pancreatic juice. He suggested that the positive results which had been obtained were the result of mechanical factors influencing the movement of the stomach and the intestine or their contents, which he believed would stimulate the pancreas reflexly. Flieg, 1903, (63) did not support the interpretation given by Popielski. He believed that after denervation, resorption from the loop stops, and that although secretin may be formed it was not taken into the blood stream. Although the experiment of Bayliss and Starling could only be considered as suggestive at the time, it should be pointed out that later workers (88–95) using a more refined technic confirmed them conclusively.

The non-ubiquitous occurrence of secretin was denied by Popielski 1902–1907 (160–161) who stated that he could prepare extracts from many tissues, blood, duodenum, jejunum, rectum, brain and even from plants which would cause the pancreas to secrete. He also pointed out that the Bayliss and Starling extract caused not only the pancreas to secrete but also the salivary glands and liver. In a long series of papers extending from 1902 to 1912 (153 to 164 inclusive) he presented many experiments to support his acid reflex theory and attempted to show that all of the secretagogue activity of secretin preparations was due to the vasodilatin contained in them. His point of view and observations may be briefly stated as follows: 1. The intravenous injection of acid, alkaline or saline extracts of many tissues and plants caused the pancreas to secrete and a marked reduction in the blood pressure. Peptone produced the same effects. 2. He observed the development of a "tolerance" reaction to repeated injections of "secretin" but no such reaction to repeated applications of acid to the intestine. 3. He found the secretagogue effect of duodenal extracts was not specific for the pancreas. He was so certain that the activity of duodenal extracts was due to such vasodilatins as histamine and other protein products that in one of his later papers he stated, "In the sense of Bayliss and Starling secretin does not exist and all the theory of secretin is in plain discord with the experimentally acquired facts." Furth and Schwartz 1909 (70) isolated considerable amounts of choline from secretin solutions and concluded that part of the secretagogue activity was due to that substance.

From time to time various authors had recorded that occasionally

they had prepared a Bayliss and Starling extract which was very potent and which contained very little vasodilatin.

It is difficult to explain how Popielski failed to note the quantitative difference in the secretagogue activity of the various tissue-extracts. Camus and Gley, 1902 (18) had shown that acid extracts of the duodenum contain much more activity than extracts of other tissues. Delezenne and Pozerski, 1906 (30) showed that unheated extracts rapidly lose their secretagogue activity due to enzyme action and it was known that the vaso-dilatin was not so affected. Henri, 1905, (85) and Divry (33) presented evidence which denied the validity of acid-reflex theory.

Zunz (211) described a method of making a dry secretin preparation which contained little or no vaso-dilatin. In 1912 Dale and Laidlow made a secretin preparation which contained very little vaso-dilatin and was a potent secretagogue. Bylina, 1911, (17) was convinced of the existence of secretin but insisted that the acid-reflex was of importance in determining the character of the pancreatic juice.

Gley, 1912, (74) found that the pancreatic juice collected after injection of secretin contained inactive trypsinogen while that collected after the injection of certain vasodilatins (drugs and peptone) contained activated trypsin. In the same year Matsuo (125) wrote, "I could not obtain secretin from other organs of the dog. Although acid extracts of various organs may give a few drops of secretion when injected into the blood stream, the action was not comparable with that of secretin."

Several attempts were made to demonstrate the presence of secretin in the blood stream by Enriquez and Hallion (56), Wertheimer and Le-Page (199) and Fleig (66–402), (also see Hedon, 80, 83) by means of injection, transfusion or cross circulation of blood from an actively secreting dog (HCl in intestine) into a non-secreting dog. They reported an augmentation in the secretion of the recipients. In Fleig's experiments the main portion of the duodenal blood from the donor was transfused into the recipient. On applying HCl to the donor's intestine he observed an augmentation in both dogs. From this he concluded that probably the acid-reflex and secretin were of equal importance in the stimulation of pancreatic secretion.

Popielski, 1902, (163) and again in 1907 (160) criticized the experiment because he found that often the injection of blood from a non-secreting dog into another would cause its pancreas to secrete.

Matsuo, 1913, (125) made a cross-circulation (carotid-jugular) between dogs. In four out of eight experiments he found that the applica-

tion of acid to the duodenum of one dog caused both pancreases to secrete. Considering the difficulties involved in carrying out such experiments his results seem very significant. Luckhardt (personal communication) using a syringe-cannula method of transfusion obtained negative results in two experiments.

It has been pointed out that the validity of Bayliss and Starling's denervated loop of intestine experiment was questioned. The specificity of secretin and its presence in the blood had not been conclusively shown. It is interesting that during the controversy between the followers of Bayliss and Starling, and of Popielski, that the latter group seemed to lose sight of their original thesis. They did not endeavor especially to advance the acid-reflex theory so much as they did to support their conviction that the potency of secretin was in its vasodilatin content. The slowly accumulating data, however, seem to have gradually given a firmer foundation to the secretin theory. When research was resumed after the war most workers accepted the secretin theory not because of undeniable proof but largely because of the large volume of suggestive data for its existence.

In 1924 Luckhardt and Blonder (121) made some experiments which emphasized certain differences between secretin and vasodilatin. They found in dogs having a very low blood pressure (transected spinal cord) that the intravenous injection of Bayliss and Starling extract provoked the characteristic pancreatic flow, without further diminution in the arterial pressure. Further, they emphasized the fact already mentioned by Bayliss and Starling (10) and by Carlson et al (21) that digestive juices destroy the secretin activity and show no corresponding action on the vasodilatin.

Barger and Dale (9) had recorded certain differences between histamine and secretin. Parsons 1925 (149) presented evidence which made untenable the theory that that element was the active substance in secretin preparation.

Ivy and Farrell, 1926, (60), (89), (94) transplanted the tail of the pancreas of the dog under the skin bringing the duct to the surface. Later the original blood and nerve supply was severed. This transplant furnished an excellent test for a humoral pancreatic mechanism. They found that food or n/10 HCl given by mouth provoked a secretion from the transplant. The fact that HCl provoked a characteristic secretion when given by mouth after 48 hours' fasting led them to conclude that a humoral and probably a hormonal mechanism was functioning. They point out, however, that their work does not afford undeniable evidence for the hormone mechanism because the intestine may have still contained

food residues, the hydrolysis of which formed absorbable secretagogues. The application of HCl to a jejunal Thiery fistula which had previously been worked out with warm salt solution gave results in confirmation with the above.

Finally these workers, Ivy, Farrell and Lueth, 1927, (95) made a jejunal loop transplant and a pancreatic transplant in the same dog. The original blood and nerve supply to both transplants had been severed. The application of water to the jejunal transplant had no effect upon the pancreatic transplant whereas the application of HCl provoked a copious secretion (see also 96).

In the same year Houssay and Mollinelli (88) demonstrated a method of anastomosing an isolated pancreas and duodenum to the vascular system of an intact dog. They found that the application of acid to the isolated duodenum resulted in secretion from both pancreases.

Necheles and Lim, 1928, (141) by vividialysis (of blood) have obtained a vasodilatin free dialysate which on intravenous injection excited the pancreas (and stomach) to secrete. The concentration of the activity was increased by either the administration of HCl or a meal.

These findings, especially those of Ivy and his co-workers, have established the physiological importance of the hormonal mechanism of pancreatic secretion. Early attempts to isolate secretin from extracts were rather unsuccessful. The methods of Zunz (211), Dale and Laidlaw (27), and Cervera (23) gave promise. Luckhardt, Barlow and Weaver 1926 (120) described a simple method of preparing a potent secretin solution which often was vasodilatin free.

In 1926 Weaver, Luckhardt and Koch (202) made a potent secretin preparation which was vasodilatin free even when injected in large amounts. In the same year Mellanby (130) by a different procedure made a very potent vasodilatin free preparation.

These findings undeniably established the physiological importance and existence of secretin. The theory of Bayliss and Starling was confirmed and the vasodilatin theory of secretin activity was shown to be essentially untenable.

Source and distribution. Little other than quantitative data has been added to the original contribution of Bayliss and Starling, 1902–03, (10–11), who showed that very little secretin could be extracted from other tissues than the mucosa of the upper small intestine. They also prepared secretin from many vertebrates (carnivora, herbivora, omnivora) and could detect no species specificity. Lalou confirmed these workers. Camus, 1906, (20) showed the presence of secretin in the intestines of guinea pig and rabbit fetuses. Hallion and Lequex, 1906,

(77) made a similar observation using the intestines from two babies who died without having taken food. Extracts of the upper intestine proved to be very active while those of the lower intestine were inactive. Pringle, 1911, (168) prepared active secretin solutions from the intestines of cat fetuses.

Mellanby and St. G. Huggett, 1926, (131) prepared by Bayliss and Starling's method extracts from different parts of the alimentary canal and injected 0.4 cc. of each filtrate into the vein of a cat with the following results:

PART OF GUT TAKEN	PANCREATIC JUICE SECRETED
	cc.
Fundus of stomach	nil
Pylorus of stomach	nil
Upper third of small intestine	2.75
Middle third of small intestine	2.6
Lowest third of small intestine	0.5
Ascending colon	0.2

Weaver, 1927, (200) found extracts of the pleura, peritoneum, endothelium of large blood vessels, lining of trachea, liver, heart, muscle, omentum, salivary glands and lymph glands to contain no secretin. He then studied the distribution of secretin in the alimentary canal in two groups of experiments. In the first he removed the intestine and divided it into six equal lengths, preparing an extract of each by the salt precipitation method. The resulting extracts were compared in the same dog. In the second group, ligatures were placed on the intact intestine so as to divide it about as it was in the first group. The effect of in vivo application of 0.4 per cent HCl to the different segments was noted. The following table is constructed from his two groups of experiments.

SOURCE	AVERAGE DROPS OF PANCREATIC JUICE	
	Extract	Acid application
Duodenum	119	27
Intestine 2	48	16
Intestine 3	18	6
Intestine 4	9	2
Intestine 5	0	0
Colon	0	0

Drewyer and Ivy, 1929, (52), using the salt precipitation method of Weaver, Luckhardt and Koch, examined brain, blood, liver, lung, kidney, pancreas, thyroid, stomach, intestine, colon, muscle, heart as well as spinach for secretin activity. None was found except in the extracts from the stomach and intestine. The mucosa of the upper intestine yielded ten times as much secretin as did the pyloric antrum.

The original findings of the non-ubiquitous occurrence of secretin by Bayliss and Starling have thus been well confirmed.

Prosecretin. Bayliss and Starling (10), because they were unable to obtain active secretin preparations by extraction by neutral solutions or alcohol, believed the mucosa to contain a substance which was hydrolyzed by acids to liberate secretin. Upon that evidence they proposed the existence of prosecretin.

Food is not necessary for the formation of secretin because it has been found in the intestine of fetuses (20), (77), (168). Nor is bile necessary for formation or activation because Camus, 1903, (19) found it in intestines of dogs having chronic biliary fistulae. Reagents which have been shown, subsequent to the work of Bayliss and Starling, to extract active secretin from the duodenal mucosa are: soap (62), chloral hydrate (59), sodium chloride solutions (30), sugar, urea, glycerine solutions (68), alcohol (138), (131), (65), dilute NaOH, phosphate solutions, and acetone solutions (131). Of the various extracting solutions acids and alcohol seemed to be the most efficient.

Gley, 1910, (71), being able to extract some secretin with saline solution and even distilled water, questioned the existence of prosecretin.

Delezene and Pozerski, 1912, (28–29) noted that unboiled extracts soon lost their activity, a process which boiling, acidification or chilling prevented, and concluded that secretin existed as such in the duodenum. They believed the preserving measures destroyed or reduced enzyme activity. Lalou, 1913, (112) also doubted the existence of prosecretin.

Starling in 1913 (175) was not sure of the existence of prosecretin. He expressed the view that secretin might be bound to the protoplasm within the cells the essential difference in this and his former views being the group to which secretin is bound.

Grogan and Luckhardt, 1924, (76) observed that on repeated applications of 0.2 per cent HCl to the duodenum of a dog, the pancreatic response gradually decreased. The duodenum from such an animal was shown to still contain secretin by preparing a Bayliss and Starling extract with 0.4 per cent HCl. This observation has been confirmed by Ivy and Javois (see 202). Grogan and Luckhardt found, however, that

the repeated introduction of 0.4 per cent HCl into the duodenum caused practically a depletion of the secretin. They pointed out the damage to the mucosa by that concentration of acid and the presence of a relatively vasodilatin free secretin solution in the duodenum, an observation which Luckhardt and co-workers used in developing an improved method of preparing secretin (202).

The views of most workers concerning the existence or non-existence of prosecretin rested upon suggestive rather than conclusive data.

Still and Keith (unpublished) have made and compared the activity of extracts prepared by the methods of 1, Bayliss and Starling (10); 2, Luckhardt, Barlow and Weaver (120), and 3, Weaver, Luckhardt and Koch (202). Many solutions were used in making these extracts and, invariably, acids gave products of greater activity than any other solution. In fact, if one destroys the cellular continuity of duodenal mucosa by freezing (CO_2 and ether) and drops the frozen mass into 1, hot 0.4 per cent HCl, and 2, hot 0.9 per cent saline, the HCl extract may contain 20 times as much activity as the saline extract. In a few cases (not a conclusive number) the acidification of the clear saline extract has increased the activity as much as 600 per cent. We have made many attempts to obtain a preparation which was absolutely inactive, but which on acidification became active. Our results have been only promising enough to keep the problem a live one. We have found nothing so potent as acid on application to the duodenum of anesthetized dogs with regard to the stimulation of the pancreas.

The question is an open one. Probably all of the secretin does not exist in free form. Whether some or all of it is held as a specific substance, prosecretin, is not known.

Preparation. Bayliss and Starling, 1902, (10) postulated the existence of secretin on the basis of the properties of an extract prepared in the following manner: the mucosa of the duodenum and jejunum was scraped off and after grinding with sand was boiled with four times its weight of 0.4 per cent HCl. The mixture was neutralized, while hot, with Na_2CO_3 and then slightly acidified with acetic acid. When cool the solution was separated by filtration. The filtrate contained much secretagogue activity and, of course, many unknown substances. Its injection caused marked vasodilatation. Enriquez and Hallion (56) and many others prepared similar extracts and found them to have the properties described by Bayliss and Starling.

In 1904 Fleig (62) discovered that after extraction of the mucosa with acid, a further extraction with solutions of soap, alcohol, etc.,

would yield more activity. He was of the opinion that each reagent extracted a different secretin and suggested the names, crinin (HCl), sapocrinin (soap), ethylocrinin (alcohol), etc., for them. Gley (73) and Stepp (176) did not agree with him and pointed out the difficulty involved in completely extracting the secretin by any means.

Most of the early work was done with secretin prepared by the original method of Bayliss and Starling or some slight modification of it. The principal effort seemed to be to determine the best solution for extraction rather than to separate secretin from the many impurities. Gley, 1910, (71) and Frouin and Lalou, 1911, (68) pointed out that strong acids were more efficient in the extraction of secretin than the weak ones.

In 1909 Zunz (211) found that extraction of the mucosa with hot alcohol removed certain impurities so that subsequent extraction with acid would yield a solution which after fractional precipitation with alcohol and ether contained little or no vasodilatin. He reported the product to contain much secretin activity. Although the procedure gave much promise apparently few workers made use of it. Probably it is to be explained by the fact that although the end product contained relatively little of the dilator substances, the secretin losses were so great as to make the method impractical.

Dale and Laidlaw, 1912, (27) prepared by a modification of the Bayliss and Starling method a new potent secretin preparation of low dilator content. This process consisted, in the main, of precipitation of the secretin together with some impurities by mercuric chloride. The mercury precipitate was extracted with acid in which the secretin was soluble. On partial neutralization most of the secretin activity flocculated and was separated. After removal of the mercury from the precipitate by passing H_2S into an aqueous-alcohol suspension, the secretin activity was precipitated by the addition of several volumes of acetone. The resulting product was further purified by precipitation with picric acid. Although their product was probably the most active preparation known at the time, it was still contaminated with dilator substances and the method was rather impractical because of the low yield.

Stepp, 1912, (176–177) dehydrated the mucosa by means of alcohol, acetone and ether. The dry mucosa furnished a convenient source of crude secretin. They extracted the crude preparation with 0.4 per cent HCl and evaporated the filtrate to dryness on a water bath. The dry residue was extracted with 70 per cent alcohol. The clear extract was

poured into 9 volumes of absolute alcohol, which precipitated a large amount of impurities and a little secretin. The filtrate was poured into one volume of ether and the resulting precipitate dried. The precipitate was soluble in saline and was of great potency and contained very little vasodilatin. He suggested the product as a starting point for further purification.

Launoy and Oechslin, 1913, (116a) precipitated the Bayliss and Starling extract with phosphotungstic acid. The washed precipitate was mixed with $CaCO_3$, extracted with warm water and filtered. The filtrate was poured into six volumes of absolute alcohol which caused a precipitate to form. The dried precipitate was soluble in water, heat stable (when dry) and an effective secretagogue.

In 1917 Gonzalez and Dalman (75) and later Dalman (29) precipitated secretin from aqueous solution by adding 9 volumes of acetone. Their product was very active and of low toxicity.

In 1925 Volbarth and Adlerberg (195) prepared an extract of the intestinal juice collected from a Thiery-Vella fistula, and found it to contain much secretin and no vasodilatin.

Penau and Simonnett, 1925, (150), making use of the accumulating knowledge of the solubility of secretin, made a very potent preparation by fractional precipitation with alcohol. They found their product behaved very much like insulin in certain respects, i.e., its precipitation by saturated salt solutions. This observation afforded them a method of further purification. Their final preparation was reported to be vasodilatin free and to be an active secretagogue in doses of 1 to 6 mgm. per kilo.

Luckhardt, Barlow and Weaver, 1926, (120) described a method of making potent secretin solutions which contained little or no vasodilatin. This method was based upon the findings of Matsuo, 1912, (125) later confirmed by Grogan and Luckhardt (76) that acid washings of the upper intestine of the living dog contained a great deal of secretin and practically no vasodilatin. Volbarth (194) made a similar observation on the secretin found in the intestinal juice from a Thiery-Vella fistula. The adaptation of Luckhardt et al. was to wash out with water the upper 5 feet of the excised intestine, clamp one end and fill with 0.4 per cent HCl. After thirty minutes the acid was removed, neutralized and filtered. This "new secretin" was a very potent secretagogue and was practically vasodilatin free. The particular advantage of the method was that the first extract was nearly vasodilatin free. Since in the hands of most workers it seemed impossible to separate secretin from

the dilatin fraction, when found together, the contribution made possible a great deal of the work which followed.

Weaver, Luckhardt and Koch, 1926, (202), using "new secretin" as a starting point, attempted to purify it further by the process used by Abel et al. in their work on pituitary hormone. In studying the effects of various reagents it was found that on saturating a solution of "new secretin" with NaCl, a flocculent precipitate formed. After washing the precipitate with saturated saline it could be dried and preserved. The dried preparation was soluble in water and was found to be a very potent secretagogue. It contained no vasodilatin when administered intravenously to dogs in doses of 60 to 100 mgm. (about 10 secretory doses). This work has been abundantly confirmed in this and other laboratories.

Weaver, 1927 (201) modified the foregoing procedure by resolution and re-precipitation of the activity instead of washing the precipitate with saturated saline. This reduced the losses of activity. The precipitate was reduced to a dry powder by alcohol, acetone and ether. The dry powder was then extracted with 95 per cent acidified alcohol in which secretin was found to be soluble. The solids were removed from the alcoholic solution by evaporation (or by the addition of 5 volumes of ether) and a white amorphous powder was obtained of great activity and which had no toxic properties.

It should be pointed out that the development of the above procedure provided an economical method of making a secretin preparation, sufficiently free from impurities as to furnish an excellent basis for further purification studies.

In the same year, 1926, Mellanby (130) published a preliminary note on the isolation of secretin, and in 1928 (132) gave the method in detail. To quote from his summary, "The method described for the isolation of secretin is based on (a) the extraction of secretin from the duodenal membrane by absolute alcohol, (b) the adsorption of secretin by precipitated bile acids, and (c) the precipitation of secretin from aqueous solution by dilute acid." His preparation was of very great potency and in small doses did not show the presence of any vasodilatin. Although no comparisons are available the product was probably the most potent prepared at the time (1926). However, Zunz and LaBarre, 1929, (212) have shown that a preparation given them by Mellanby caused vasodilatation and hypoglycemic effects when injected in dogs in doses of one milligram or more. Mortimer and Ivy, 1929, (138), in a critique on Mellanby's method point out the very small yield and the unreliability

of certain steps in his process. I have repeated Mellanby's method on several occasions and came to the same conclusion as Mortimer and Ivy. In fairness it should be pointed out that probably his low yield is due to the original extraction with alcohol and that his method applied to concentrates (such as the Weaver product) works very satisfactorily. However, the end product is no more active than that prepared by the methods to be described later.

Takacs, 1928, (182) (183) described a method of preparing secretin from his concentrates making use of fractional precipitation and ultradialysis. Although his preparations were very active he found them to contain other physiologically active substances. Therefore, his work has not essentially contributed to the problem of the isolation of secretin.

Still, 1930, (180), using the Weaver, Luckhardt and Koch concentrate as a starting point, removed the bulk of the inactive material by reëxtraction with dilute acid and precipitation with trichloracetic acid. The resulting active fraction was extracted with 90 per cent acid alcohol in which most of the activity was soluble. Much of the remaining inactive material was precipitated by adding brucine and the base pyridine to the solution. The active material was then precipitated by the addition of ether. A further extraction of the dry substance with methyl alcohol (an excellent solvent for secretin) and precipitation by ether gave a product which after the removal of the small amount of remaining brucine was very active. This product was shown to be free from vasodilator substances, choleocystokinin, oxytocic substances and hypoglycemic substances.

Ivy, Kloster, Drewyer and Lueth (102) extracted the salt concentrate twice with 95 per cent ethyl alcohol, in which most of the secretin was soluble. The filtrate was evaporated to dryness and the residue taken up in water. On adjusting the pH to 5.3–5.7 a precipitate formed which was filtered off. They precipitated the secretin from the clear filtrate by the addition of trichloracetic acid to 5 per cent. The precipitate was dried by washing with acetone and ether. They called this product S-I. On extracting the pH precipitate with 0.4 per cent HCl they obtained a solution which contained considerable secretin. This was removed by trichloracetic acid (as in S-I) and dried with acetone and ether. They called this product S-II. They suggested that these two products were different secretins. They gave no data to support the suggestion other than the difference in solubility noted above. It is entirely possible that the two activities are the same, and that the solution was saturated at the pH 5.3–5.7. There seems no need for postulating the existence of more than one secretin from the data they present.

Doctor Ivy and I have exchanged products and according to our independent assays have found them to be of about equal potency.

A summary of the properties of secretin taken from the above papers is given below:

	MELLANBY	IVY	STILL
Total nitrogen		7.5%	6.7–8%
PO_4	+	−	−
S	+	+	+
Biuret	+	+	?
Xanthoproteic	+	−	−
Millon	+	−	−
Pauly	+	+	?
Ninhydrin	−		−
Molisch	−	−	−
NH_2 (Van Slyke)	−		
Hopkins-Cole		−	−
Pettenkofer		+	−
Ash		0.8	
Hypoglycemic substances	−*		−†
Liver stimulation	+	+	+
Choleocystokinin	Not tested	−	−

* Not confirmed.
† Confirmed.

Most of the earlier workers considered secretin to be protein in character, because the crude extracts contained large amounts of protein and all of the records of chemical analyses show a nitrogen content of about 15 per cent. In all cases the specific and non-specific protein and amino acid tests were found to suggest a protein character. With further purification however it became evident that it probably was not a protein. Mellanby (131) found it was not precipitated from solution by half saturation with $(NH_4)_2SO_4$, but was precipitated by full saturation with that salt. This and other properties caused him to conclude it to be a secondary albumose. On further purification, the product retained the above characters and he (132) suggested that it was a polypeptid having a high phosphorus content. No doubt the precipitation by neutral salts and the solubility in strong alcohol led him to that conclusion.

Luckhardt (119) and later Still attempted to separate secretin from the vasodilatins and other impurities by dialysis. Collodion membranes were used and it was found that although the rates of dialysis of secretin

and some of the impurities were different, the method was not practical for the purification of secretin. Takacs used a membrane which would not permit Congo red to pass and was able to retain the secretin within the dialysis sac. He reported the process to separate all of the phosphorus compounds from the secretin.

Ivy and his co-workers, and Still have found their crude products to contain about 15 per cent of nitrogen, which in the light of the foregoing suggests that secretin is similar to a polypeptid. However, on further purification the nitrogen content decreased by approximately 50 per cent. In a specific case Still found the following: The crude material, *A*, contained 14.2 per cent nitrogen and 0.9 per cent phosphorus; a fraction, *B*, prepared from *A* contained about 80 per cent of the activity of *A* and had 7.81 per cent nitrogen and no phosphorus. The final preparation, *C*, (made from *B*) contained 6.66 per cent nitrogen and no phosphorus. Per unit of weight *C* was about 200 times as active as *A*. The crude material *A* gave all of the characteristic protein reactions, while in the final product, *C*, the reactions were either negative or weakly positive. The final products reported by Ivy and Still are white and amorphous. On drying in air they tend to darken. These products are much more soluble in acid or alkaline solutions than in distilled water, which indicates that secretin is an amphoteric substance.

In view of the above properties of secretin it is certain that the molecule is not a particularly large one. The specific amino acid tests indicate that tyrosine, histidine, tryptophane, and phenylalanine are not present in the molecule, and amino acids have not been isolated from secretin. It has been shown (Mellanby) that free α NH_2 groups are not present in the molecule. Thus one must question the belief that secretin is a polypeptid.

Although the attempts to obtain crystalline secretin have been without success, it is reasonable to expect that the final criterion of purity of this compound should be its isolation in crystalline form. To this end workers in the field are busying themselves.

Secretin, per se, has been shown to be a hormone whose most characteristic activity is associated with the digestive organs; particularly the secretion of pancreatic juice and bile. That secretin has no effect on gastric secretion or the visceral vasomotor state became evident with the improved methods of making more purified preparations. The work of Weaver (203–204), who used the vasodilatin free product of Weaver, Luckhardt and Koch, seems particularly conclusive in that connection. Adequate data are not available concerning the effect of secretin on the motor and secretory activities of the intestine.

ASSAY. The only published procedure for the assay of secretin is that of Ivy et al., 1930, (102). They determined the threshold dose, which they define: ". . . that amount of dried material in solution which when injected will cause a 10 drop (0.4 cc.) increase in the rate of flow of pancreatic juice within a ten minute period following the time of injection as compared with the preceding ten minute period."

The author (unpublished) has adopted the following procedure: We have a potent vasodilatin free preparation which has been preserved as our *standard* and to which all preparations are compared. We have found that 4 mgm. administered intravenously (dog) will cause the pancreas to secrete at about 50 per cent of its maximum rate. We make all injections when the rate of the pancreatic flow is 1 drop in 4 minutes. The standard (4 mgm.) is injected and as soon as the rate returns to 1 drop in 4 minutes the unknown is injected (in such quantity as to be nearly equal to the standard) and followed by another dose of the standard. The first and last standards are usually within a few drops of each other. We then calculate as follows: $\frac{\text{no. drops unknown}}{\text{av. no. drop. standards}}$. This gives the number of units in the amount of unknown injected.

Neither of the above methods provide as accurate a method of assay such as is available for some other hormones. However, they enable one to follow the effectiveness of purification processes.

BILE FORMATION. In 1880 Rutherford (169) showed that the application of acid to the duodenum caused a marked augmentation in the flow of bile. This work did not arouse much interest until Bayliss and Starling (1902) showed that the intravenous injection of duodenal extracts caused a similar effect. These observations were abundantly confirmed (86) (32) (145) (147) (42) (41) (142). Fleig 1904 (64) (61) applied acid to the mucosa of a loop of intestine and injected blood from it into another dog. There followed an increase in the flow of bile.

Okada observed that the intravenous injection of secretin preparations into animals having biliary fistulae led to a doubling in the flow of bile. He found, however, that if the secretin had lost its pancreatic activity, it no longer stimulated the flow of bile.

The observation that the stimulation of both, bile flow and pancreatic flow, diminished as one applied acid to lower levels of the intestine coupled with the above findings were evidence that secretin exerts a cholagogue action. However, it should be pointed out that the secretin preparations used contained much unknown material.

In 1928–30 Mellanby (128), Lueth and Kloster (123) and Still (180)

reported that highly purified secretin which was vasodilatin-free caused an augmentation in the rate of bile flow. Mellanby was attracted by the long latent period as compared with the latent period for the pancreas. After a careful study of the mechanism in which he tied off the pancreatic vein to prevent pancreatic blood from returning to the circulation, he came to the conclusion that metabolites from the active pancreas were the cholagogues involved. His work suggests that we can no longer consider secretin, per se, to be a biliary excitant. However, the question is not to be considered settled because of the following: Mellanby's explanation is in accord with the observations on the effect of acid in the intestine on bile and pancreatic flow, each diminishing as the acid is applied at lower levels. It is not in accord, however, with Fleig's (61) observation on transfusion of blood from a secreting dog to a non-secreting dog. I have not been able to confirm Mellanby, using the dog. Even in depancreatized dogs secretin greatly increased the flow of bile. The possibility, however, still remains that metabolites from some other tissue (particularly the intestine) may be the factor which acts as a cholagogue. This possibility is being investigated. Takacs (181) injected large doses of secretin prepared by his method and observed no cholagogue effect in dogs with biliary fistulae. His results suggest that possibly he has eliminated an impurity from his secretin which was present in the preparations used by Mellanby, Lueth and Kloster, and Still.

The mechanism of the cholagogue action of secretin is an open question.

THE GALL BLADDER. In 1915–16 Okada (146) observed that the ingestion of a meal or the application of HCl to the intestine increased the tonus rhythm of the gall bladder. Braga and Campos, 1919 (15) reported that the injection of crude secretin preparations caused the expulsion of bile from the gall bladder.

Mellanby, 1926 (126) reported that a strip of gall bladder suspended in oxygenated Ringer's solution could be caused to contract by the addition of a trace of "pure secretion."

Ivy and Oldberg, 1927–28, (92), (93), (97) demonstrated the presence, in secretin preparations, of a motor hormone to the gall bladder which they called "choleocystokinin." In a conclusive series of experiments Ivy and his co-workers Oldberg, Kloster, Lueth, and Drewyer (122) (98) (99) (100) (101) (90) have practically separated secretin from choleocystokinin and have shown them to act independently and specifically. Their best preparations in doses of about 1 mgm. will cause a vigorous increase in the tone rhythm of the dog's gall bladder.

Drewyer and Ivy, 1930, (53) have shown choleocystokinin to be present in the intestinal mucosa of man as well as animals.

They have confirmed their findings in experiments in which the extract was injected intravenously and by cross circulation experiments. The application of HCl to the intestine of one dog caused the gall bladder of the other dog to react.

The in vitro experiments of Mellanby are interesting in contrast to the findings of Halpert and Lewis 1930 (78) who found that Ivy's choleocystokinin had no effect on the excised gall bladder of the dog.

Little is known of the chemistry of choleocystokinin.

Effect on composition of pancreatic juice. Zilwa, 1904, (205) noted that the pancreatic juice from secretin stimulation was of higher alkalinity and lower protein content than that from pilocarpine stimulation. Morel and Terroine, 1909, (137) observed a diminution of alkali and lipase content of the pancreatic juice after repeated injections of secretin. Braga and Campos, 1919, (15) found that the composition of the pancreatic juice varies with different methods used in preparing the secretin.

Dubois and Polonovski, 1925, (54) found the pH of pancreatic juice after eserine or pilocarpine injection to be lower than in juice after secretin injection. Mellanby, 1925, (129) found (urethanized cats) the pancreatic juice secreted after the intravenous injection of secretin to contain a constant quantity of $NaHCO_3$, about 0.14N but diminished enzyme content, with continued secretion. Stimulation of the vagus nerve increased the enzyme content therefore the diminution was not due to exhaustion. Finding that removal of vagal control by section or atropine did not diminish the alkali content nor the rate of secretion, he advanced the hypothesis . . . "That the enzyme content of the pancreatic juice is determined by the vagus nerve, whereas the quantity of bicarbonate solution in which these enzymes are contained is determined by secretin. On this hypothesis, secretin may play a subsidiary part in pancreatic digestion, since it only insures the presence of an optimal reaction for the activity of the pancreatic enzymes."

Still and Barlow's findings, 1927, (178) in part were in accord with the findings of Mellanby. They found the more purified secretins to cause a flow of pancreatic juice of low specific gravity and low trypsin content, while vagal stimulation (pilocarpine) caused a juice of high solid and enzyme content to be formed.

Rasenkow, 1929, reports the interesting finding that secretin injections after vagotomy produce an active (enzymically) juice, whereas before vagotomy the juice is inactive.

In the main most workers agree that the pancreatic juice obtained from secretin injections (especially since purified secretin has been available) is of relatively high alkali content and corresponding pH value. The enzyme and solid content rather parallel one another and are relatively low as compared to vagal juice (nerve stimulation or drug). Mellanby's analysis of the question seems to be an excellent one. Interesting papers in this connection by Ball and by Johnston, 1930, (103) (8) (7) throw a somewhat new light, however, upon the question. They found that the composition of the juice rather follows the composition of the blood and that the rate of secretion was an important factor in determining the composition of the juice.

CARBOHYDRATE METABOLISM. Numerous investigators have associated the internal and external secretions of the pancreas. Moore, Edie and Abram in 1906 (134), (135), (136) observed beneficial effects of the administration of duodenal extracts to a small series of diabetics. Bainbridge and Beddard (5) (6) being unable to confirm them suggested the beneficial effects (mainly reduction in urinary sugar) was due to dietary measures. Moore et al. collected data from a larger series of cases in which the dietary factor was controlled and obtained results in confirmation to their earlier work.

Pariset, 1906, (148) found that the injection of secretin preparations into the portal vein did not effect mobilization of carbohydrate from the liver.

Foster (67), and Dakin and Ransom (26) injected secretin into diabetics and obtained small reductions in urinary sugar. However, these workers were not enthusiastic over the therapeutic possibilities of secretin.

Murlin, 1913, (139) found that the intravenous injection of secretin prepared according to Knowleton and Starling caused a decrease in the D/N ratio in diabetic dogs. However, they were unable to obtain evidence of altered carbohydrate metabolism as measured by the R.Q. In 1914 Loeb and Stadler (117) were unable to show any influence of crude secretin on carbohydrate assimilation.

Abelous and Saula, 1920, (1) injected crude secretin into dogs and obtained data which indicated an increased carbohydrate oxidation (the R.Q. rose from 0.75 to 0.91) and a rapid mobilization of carbohydrate (blood sugar increased from 140 mgm. per cent to 190 mgm. per cent; and the liver glycogen decreased from 2.8 per cent to 0.95 per cent).

Oehme and Wimmers, 1923, (143) found that intravenous injection

of crude secretin preparations reduced the blood sugar of fasting dogs below the fasting level.

Santos and his co-workers in a series of papers (170) (171) (172) (173) found that intravenous injections of secretin preparations caused a small decrease of the blood sugar. They were, however, unable to confirm Penau and Simonnett (150) (151) who found secretin effective in depancreatized dogs. Therefore, they concluded the effect was due to stimulation of the islet tissue by the secretin. Troteano came to the same point of view (191). Santos' work was amplified and confirmed by Criado in 1928 (25).

Ivy and Fisher, 1924, (91) obtained a product from the mucosa of hog duodena and stomachs, by the Shaffer-Fisher procedure for preparing insulin, which was a potent hypoglycemic agent. Their attitude seemed to be that the product contained insulin, per se, which had been extracted from the intraduodenal insular tissue which has been shown to exist in many animals.

Lambert and Hermann (113) (87) (114) and Tournade and Hermann (189) believed the hypoglycemic properties of secretin containing extracts were due to effects on the adrenals. They found purified secretin to have no effect on blood sugar.

Penau and Simonnett, 1925, (50) separated from duodenal extracts a preparation which was a potent secretagogue, non-hypotensive and which was hypoglycemic even in depancreatized dogs.

In 1927–28 Takacs (181) (182) (183) (184) (158) (186) (187) described a method of making a secretin preparation which reduced the ketosis in diabetic patients and reduced the blood sugar in normal or diabetic rabbits, dogs or man. He found his preparation to be effective when administered by mouth, rectum or by intravenous injection. Tangle and Than (188) and Heller (94) were in agreement with Takacs and affirm the belief that the extracts are "effective in the care of normal or pathological blood sugars."

In 1928 Mellanby (132) found that the subcutaneous injection of his very highly purified secretin had no effect upon blood sugar. However, Zunz and La Barre (212) using a product given them by Mellanby and Underhill observed a marked hypoglycemic effect on intravenous injection in dogs.

Gley and Hazzard, 1928, (72) observed that the transfusion of blood from a dog having a strong acid solution in its intestine into a normal dog caused the blood sugar of the latter to be diminished. Their experiment indicates that either a substance is liberated and absorbed from

the intestine which is hypoglycemic itself or the islet tissue is stimulated to produce more insulin.

Zunz, La Barre and Destrée have recorded correlations between the internal and external secretions of the pancreas (214). Zunz and La Barre, 1929, (206) by means of a pancreatico-jugular venous anastomosis between dogs (the receptor being adrenalectomized) were able to show that the injection of dilatin-free secretin preparation caused a marked stimulation of the islet tissue and a subsequent diminution of the blood sugar. This diminution did not occur if the vagi were cut (207).

In a group of excellent experiments (206) (207) (208) (209) (210) (212) these workers, using various highly purified secretin preparations, showed that the preparations were hypoglycemic by virtue of the stimulating effect upon the islet tissue. They did not agree with former workers who believed the hypoglycemia was caused by included insulin.

Still and Shpiner, 1930, (179) studied the effect of purified secretin on the blood sugar of normal and depancreatized dogs and were unable to show any change even in cases where 50 or more secretory doses were administered intravenously. Doctor La Barre, visiting at the University of Chicago, kindly consented to repeat his experiments using the author's secretin preparation. It was found that the secretin had no effect upon the blood sugar, even in adrenalectomized dogs (109–110). Our work indicated that the residue from which secretin has been extracted contains two substances which affect the blood sugar. One stimulates the islet tissue and the other acts very much like insulin except that it is more stable to certain reagents. The former, in the light of the work of Zunz and La Barre, seems to be well established; the latter is being further investigated.

Zunz (213) independently showed that a purified secretin preparation (made by Penau and Simonnett) had none of the hypoglycemic effects.

It seems certain that secretin, per se, has no effect upon sugar metabolism. The hypoglycemic fraction or fractions, however, have been partly separated and in the light of Takacs' findings should be carefully investigated. The therapeutic possibility is obvious. It might be stated that I have tried to prepare a residue similar to the one used by Doctor La Barre and me which would be hypoglycemic when administered by mouth. My results have been negative. The effect of purified secretin on the respiratory quotient has not been studied.

Effect on blood. In 1917 Downs and Eddy (47), reported that an optimun dose of 1 cc. of Bayliss and Starling's secretin per kgm. (rabbit)

caused a prompt but transient increase in the RBC per cmm. The following year (50) they reported a similar effect on the white blood cells.

Fujimoto, 1918, (69) confirmed the findings of Downs and Eddy and added that the secretin increased the catalase content of blood but was without effect on the diastase, glycolytic enzyme or sugar content.

Downs and Eddy, 1918, (44) reported that the increased red and white cell counts of rabbits after subcutaneous injection of secretin were dependent upon new cell production. They based their opinion upon post mortem examination of the bone marrow and lymph glands. Further, these workers (43) found an increase in the cell counts during digestion similar to the increase from the injection of Bayliss and Starling's secretin. The differential leukocyte counts were similar in both cases and they attributed the effect in digestion to the absorption of secretin.

They pointed out in 1921 (48) that subcutaneous injection of extracts of the duodenum (thus limiting the name secretin in their future work) caused a more rapid improvement of anemic animals than diet alone. This was shown by increases in the rate of blood formation and of growth. (Previously, 1919, Voegtlin and Meyers (192) (193) came to the conclusion that vitamine B (the complex) and secretin were similar if not identical because of the following findings: 1. The two react in a similar way to such reagents as MeOH, Ag, Pb and Ba. 2. Secretin preparations relieve polyneuritic symptoms. 3. Injection of vitamine B preparations causes pancreatic secretion. 4. The two properties (2 and 3) were destroyed by the same procedures, and 5, an extract of the duodenum of a polyneuritic cat had no influence on pancreatic secretion. Cowgill, 1921 (24), Anrep and Drummond, 1921, (3) and Downs and Eddy, 1921, (48) disproved conclusively the view of Voegtlin and Meyers.) Therefore, Downs and Eddy concluded the beneficial effects were not due to vitamine B.

In a series of papers (45) (46) (49) Downs and Eddy report the effect of duodenal extracts on the anti-bodies of the blood. They injected one group of rabbits with sterile cultures of B. typhosus and secretin (dried acid extract of duodenal mucosa) and treated a control group in a similar manner except they received no secretin. They found that the rabbits receiving the secretin had higher agglutinin and hemolysis titres than the control animals. They observed no change in the complement titre. Similar studies have not been carried out with purified secretin preparations and although it is not known just what substance

was responsible for the effects observed one can not conclude that secretin, per se, had anything to do with the phenomena.

Skarzynska, 1924, (184) reported that she could produce no immunization against secretin preparations.

King (104) (105) denied that secretin preparations were hematopoietic stimulants. He found the effect to be rapid and transitory and could be accounted for by the concentration of the blood rather than by new cell formation.

Matsumoto, 1927, (124) found the intravenous injection of (crude) secretin preparations to increase the blood platelets.

Kuzwanek, 1929, (108) believed the increase in the erythrocyte count following the injection of secretin preparation to indicate increased splenic activity.

In the light of our more recent knowledge concerning the metabolism of minerals one wonders if the improvement of the blood and growth of animals with nutritional anemia was not in part at least due to the minerals contained in the extracts used by Downs and Eddy. It would be of interest to know if any of the blood changes recorded above would be obtained using the highly purified secretin now available.

THERAPEUTIC VALUE. From time to time secretin has been suggested as an agent of therapeutic value. Harrower, 1913, (79) advocated secretin therapy in gastro-intestinal disorders. In 1916 Carlson (21) (22) stated, "Secretin therapy at the present state of our knowledge is a fraud." Prentiss (165) (166) (167) suggests secretin to be a defensive factor against gastro-intestinal cancer; Downs and Eddy, 1923, (51) suggest its clinical application as a hematopoietic stimulant and for improvement of nutrition.

Other therapeutic suggestions are considered elsewhere. At the writing of this review, the author is not aware of any proven therapeutic value of secretin, per se. However, one should not lose sight of the other suggested activities of substances found in the duodenum, for it is not improbable that some of them, when properly isolated, may prove to be of inestimable value.

KIDNEY. Cow in 1914 (23a) reported that acid extracts of the duodenal mucosa acted as stimulants to the kidney. Piticarin, 1916 (152) confirmed his findings but added that the extract must be prepared from the same species. Neill and Fritz, 1926, (142) observed after injection of secretin preparations first there was a diminished flow of urine followed by diuresis (relation of blood pressure). Owen and Ivy, 1930, (144) in conjunction with their studies on secretin found vasodilatin

free extracts of the intestine cause a diuresis which they believe is caused by a specific substance.

FATE OF SECRETIN IN DIABETES. In 1912 (57) Evans reported that secretin disappeared from the intestine of depancreatized animals. Bainbridge (6), Bainbridge and Beddard (5) Hedon and Lisbonne (81) and Nakagawa and Harado (210) did not confirm his findings. Finally Hedon, 1929 (82) found secretin in the duodenun of a dog 15 months after excision of the pancreas.

MISCELLANEOUS. Many workers have found secretin to cause a flow of saliva (116). Probably this was caused by some of the unknown impurities in the earlier extracts because most of the more recent workers (142) have not found secretin to influence the salivary glands. In this connection, Ivy and his students have extracted many tissues including the membranes of the buccal cavity, and have been unable to obtain any evidence of the existence of a secretory hormone to the salivary glands.

Eddy and Downs (55) found secretin preparations to retard the onset of fatigue and to increase the work done by skeletal muscle. Abelous and Soula, 1920, (1) found that the intravenous injection of a crude secretin preparation or the application of HCl to the duodenum caused an increase of urinary nitrogen and a more rapid autolysis of excised organs. Troteano, 1924, (190–191) found that crude extracts increased the nitrogen metabolism of the turtle's heart as measured by the increase in the non-protein nitrogen.

Barbera, 1906, (9a) fasted dogs until they had lost 45 per cent of their weight and found their intestines to still have the normal amount of secretin.

Alessandro, 1912, (2) reported crude secretin to stimulate the flow of lachrymal fluid.

Bottazzi, 1903, (14) and Mendel and Thacker (133) observed an increase in the lymph flow after injections of crude secretin.

Barlow (9b–9c) studied the organ volume, general and splanchnic vascular changes, and pancreatic secretion rate after intravenous injections of various vaso-dilatins and secretins.

Secretin has been reported to stimulate the secretion as well as the peristalsis of the intestine.

Mellanby (132) has shown that his purified secretin causes a marked stimulation of the rhythmical contractions of isolated strips of intestine suspended in oxygenated Tyrode solution. Templeton (personal communication) has injected my purified secretin intravenously into dogs prepared for the study of gastro-intestinal movements and has not been

able to show any effect. He has, however, found that the residue (after extraction of secretin from the salt concentrate) contains a substance or substances which cause an immediate augmentation in the contractions. It is not known whether the activity is due to a specific substance or to a cleavage product formed in making the extract. He is studying the problem and trying to determine whether it is of a physiological or pharmacological nature.

It seems probable that secretin augments the flow of succus entericus. The question has not been studied by workers using purified secretin. The following observations, however, are very suggestive. We have prepared numerous dogs with chronic pancreatic fistulae (after Pavlov) and have found that the intravenous injection of 1 milligram of our purified secretin causes the production of from 5 to 15 cc. of pancreatic juice in twenty or thirty minutes. Doctor Dragstedt has recently developed a new technique for making chronic pancreatic fistulae. The common bile duct is transplanted to the stomach or jejunum. A Thiery fistula is then made of that part of the duodenum to which the pancreas is attached. On injection of 1 mgm. of the purified secretin, over 40 cc. of fluid were collected in thirty minutes. It seems more than probable that the "extra fluid" was succus entericus. A detailed study of the question has not been made.

PLANT SECRETIN. Many authors have described the preparation and properties of "plant secretin." These preparations almost invariably possess potent vasodilatins and probably are not to be considered similar to pancreatic secretin (36) (35) (31) (37) (107) (38) (58) (12) (13) (34).

CONCLUSION

A great deal of interesting and valuable information has come from investigations on secretin. Of greatest interest to me is the opening of a field for investigation which may make for great advances in the knowledge of digestion and metabolism. It has been suggested that the duodenum contains in addition to secretin, humoral or hormonal substances which stimulate the kidney, liver, gall bladder, the islet tissue, and possibly the motor and secretory activity of the intestine. The further study of these substances should add greatly to our knowledge.

The secretin theory has been proven conclusively. The quest of the crystalline substance and its synthesis remain. The mechanism of secretin stimulation of the pancreas is not understood. Secretin,

per se, seems to stimulate the pancreas, and probably the liver and the duodenal mucosa. Other physiological effects have not been proven.

I have tried, in the limited space, to present the data and interpretations from the more conclusive and primary papers.

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THE MODE OF ACTION AND DIFFERENTIATION OF PROTEOLYTIC ENZYMES

ERNST WALDSCHMIDT-LEITZ

Institut für Biochemie, Deutsche Technische Hochschule, Prag

The possibility that a chemical combination between enzyme and substrate led to the phenomenon of enzymic specificity, was first suggested by Emil Fischer (1). He illustrated this view with the well-known analogy of lock and key, which carries with it the assumption that the compound formation is dependent not only upon chemical, but upon stereo-chemical relationships.

The subsequent development of colloid chemistry pushed this simple and clear-cut conception somewhat into the background. An attempt was made, especially by W. M. Bayliss (2), to explain enzyme action as a surface phenomenon. According to this view, the enzyme particle adsorbed the substrate, whose decomposition was therefore accelerated at the surface of the enzyme, due to its increased concentration there, and the resulting increase in reaction-velocity. This conception has today been abandoned. It has always lacked experimental proof, and does not appear highly probable. On the other hand, modern investigations of the mechanism of adsorption no longer permit so strict a differentiation between adsorptive and chemical combination. The modern conception of enzyme-action, which has already withstood considerable experimental testing, is, in its essential features, a continuation of the thought of Emil Fischer.

One of the most important supports of the recent chemical explanation of enzyme-reactions comes from the experiments of L. Michaelis and M. L. Menten (3) on the hydrolysis of saccharose by yeast-invertin. It is here shown that the enzymic scission of the cane-sugar conforms to the law of mass-action, when it takes place in a homogeneous solution.[1] The entire amount of substrate present, not an adsorbed fraction of it, is responsible for the velocity of the reaction. The affinity of the saccharase for its substrate may be numerically expressed, and is independent of the degree of purity of the enzyme (5). Similar results have been

[1] For arguments against this view, see R. Weidenhagen and E. Landt (4).

obtained with other ferments (6). And even if these results do not enable a definite decision for a purely chemical reaction between enzyme and substrate—as against an adsorptive process—they have nevertheless led to a thorough investigation of enzyme-action from a chemical standpoint.

In other cases, however, especially with the enzymic hydrolysis of fats, experience has shown that the ferment-activity is to some extent dependent upon the degree of dispersity (7) of the enzyme. The splitting of water insoluble fats by the water soluble animal lipase, for example, appears to be preceded by an adsorption of the fat onto colloidal particles already bearing the enzyme. From this situation arose the now generally[2] accepted conception of Willstätter (9), which in a sense unites the contradictory views of Bayliss and of Michaelis. Willstätter regards the enzymes as composed of a colloidal carrier and one or more active groups. The latter are responsible for the reaction between enzyme and substrate, but their extraordinary activity is dependent upon their connection to the colloid.

The enzyme reaction, therefore, takes place through an intermediary enzyme-substrate compound. The formation of such a compound and its subsequent disruption into free enzyme and the split-products may, according to Michaelis, be analytically differentiated. The two steps of the reaction—the formation and the decomposition of the enzyme-substrate compound—may be influenced to different degrees by added substances, for instance, substances inhibiting only one of the steps. According to the so-called "two-affinities theory" of H. v. Euler and K. Josephson (10), these two steps require two different active groups of the enzyme molecule, a substrate-binding and a substrate-splitting group. These correspond to two different enzyme-reacting groups in the substrate. The splitting of the substrate therefore takes place at some point other than that to which the enzyme is attached. The modern theory of enzyme-action thus resembles to a remarkable degree the "side-chain theory" of Paul Ehrlich (11), with its distinction between haptophore and functional groups. As a matter of fact, the nature of the groups both in substrate and in enzyme, which take part in some examples of peptid hydrolysis, can now be described. The considerations leading to these results are detailed in the following paragraphs.

The proetolytic enzymes are divided, according to their specificity, into the two large groups of proteinases and peptidases. The action of the proteinases is, as the name implies, confined to the splitting of pro-

[2] Compare the views of A. Fodor and L. Frankenthal (8).

teins. They are without effect on the synthetic peptides, while the peptidases, which split these, have not been observed to attack the natural proteins. The proteinases are again subdivided into the pepsinases, the tryptases and the papainases, which are distinguished by their different ways of attacking the protein molecule. According to J. H. Northrop (12), the pepsinases, for example pepsin, react with the protein cations, while the tryptases, among them pancreas trypsin, react with the protein anions. According to R. Willstätter and W. Grassmann (13), the ferments of the papainase group react only with the isoelectric protein.

The sub-division of the peptide-splitting enzymes rests, analogously, upon differences in their mode of attacking the peptide molecules. They are divided into carboxy- and amino-polypeptidases, dipeptidases, and

TABLE 1

Composition of several proteolytic enzyme-systems of different origins

PANCREAS-INTESTINE (15)	GLANDULAR TISSUE (16)	YEAST (17)	WHITE BLOOD CORPUSCLES (18)
Pancreas-proteinase (tryptase-type)	Cathepsin (papainase-type)	Proteinase (papainase-type)	Proteinase (tryptase-type)
Protaminase (19) (tryptase-type)			Cathepsin (papainase-type)
Carboxy-polypeptidase	Carboxy-polypeptidase	Carboxy-polypeptidase (20)	Carboxy-polypeptidase
Amino-polypeptidase	Amino-polypeptidase	Amino-polypeptidase	Amino-polypeptidase
Dipeptidase	Dipeptidase	Dipeptidase	Dipeptidase
Prolinase (21)	Prolinase (21)	Prolinase (21)	

imino-peptidase or prolinase, according to the structural characteristics of the peptides or peptide derivatives which are necessary for the attack of the ferment.

In nature the above enzymes occur for the most part in mixtures, in which the effects of the single enzymes cannot be recognized and differentiated. The isolation of these different types of proteolytic enzymes, and the possibility of distinguishing between them, is due to the application of new methods of preparation. Most important among these methods is the process of adsorption and elution, introduced into enzyme-chemistry by R. Willstätter (14).

The composition of some of the better known naturally occurring enzyme mixtures is shown in table 1. Here are tabulated, as far as can be done today, the enzymes occurring in the proteolytic systems of the

animal intestinal tract, glandular tissue, white blood corpuscles, and yeast.

The separation of such enzyme-mixtures has been made in a number of instances, of which the separation of the pancreatic enzymes will be here discussed as an example. The separation of these enzymes, as they occur in extracts of the pancreas gland, begins with the adsorption of the mixture with the alumina-gel C γ (composition $Al(OH)_3$) by acid reaction (pH 4) (22). The liquid portion after adsorption contains proteinase, protaminase and carboxy-polypeptidase; the adsorbate contains amino-polypeptidase, dipeptidase and prolinase, which may be obtained from the alumina by elution with alkali. The two simpler mixtures resulting from this adsorption are farther separated by other adsorption operations (23). These are indicated in the scheme shown below:

Proteinase, Protaminase, Carboxy-polp., Amino-polyp., Prolinase, Dipeptidase
+ Alumina-gel Cγ (at pH 4)

Solution after adsorption: Proteinase, Protaminase, Carboxy-polyp.	Elution of Adsorbate: Amino-polyp., Prolinase, Dipeptidase
+ Alumina-gel Cγ (at pH 7) Solution after adsorption: Proteinase, Protaminase, Carboxy-polyp.	+ Iron hydroxyde (at pH 4) Solution after adsorption: Elution: Amino-polyp., Prolinase, Dipeptidase

The adsorptive behavior of the proteolytic enzymes is to a remarkable extent independent of their degree of purity. It does not appear to be influenced by accompanying substances, and seems to be a property of the enzymes themselves.

The adsorption in these particular cases may be regarded as an electrochemical reaction between enzyme and adsorbent, in the sense employed by L. Michaelis (24). The most selective adsorbents are not the gels with large specific surfaces, but are crystallized oxyhydrates and oxides, in many cases minerals (25).

Our knowledge of the several protelytic enzymes has been also increased by a study of the properties and modes of action of certain auxiliary substances, the specific activators. The enterokinase of the intestinal mucosa (26) functions as a specific auxiliary substance for the proteinase and the carboxy-polypeptidase of the pancreas. It forms with these enzymes a dissociable addition-product. Without the acti-

vator the enzyme is partially active in the case of carboxy-polypeptidase (27), and totally inactive in the case of the proteinase.

A similar activation is undergone by the enzymes of the papainase group (for example, papain (28), or kathepsin), and by the carboxy-polypeptidase of animal tissues and organs (29). This activator, called phytokinase (30) or zoökinase (29), according to the source of the material, is frequently associated with the enzymes, and its formation can also be brought about by the action of hydrocyanic acid or hydrogen-sulphide upon impure preparations of the ferments (31). The nature of this activator is known. It is identical with glutaminyl-cysteinyl-glycine (32), the reduced form of glutathione. Only the sulphydryl, and not the disulphide form of glutathione is capable of activating pro-

TABLE 2

Enzymic decomposition of proteins

(+ = hydrolysis; − = no hydrolysis)

	PEPSINASE	PAPAINASES (ACTIVE)	TRYPTASE (ACTIVE TRYPSIN)	PROTAMINASE	PEPTIDASES
Proteins:					
Casein, histone, fibrin, gelatine, gliadin, zein, albumin, globulin..	+	+	+	−	−
Protamines:					
Clupein, salmin, scombrin.....	−	+	+	+	−
Skleroproteins:					
Silk-fibroin, keratin.....	−	−	−	−	−

teolysis (33). It is thus clear that there is an important connection between the oxidation potential in the cells, and the intracellular proteolytic activity. The commencement of autolysis at the death of the cell is thus easily accounted for, as well as in tumor tissue, where it is the result of a reduced respiration (34).

As far as we know, the action of all the proteolytic enzymes, proteinases as well as peptidases, consists in the splitting of acid-amide linkages, the −CO−NH− bonds as they occur in the peptides (35). The study of the specificity of these enzymes has nevertheless shown that they do differ in their modes of action. Before this can be farther discussed, however, the action of the enzymes on both naturally-occurring and synthetic substrates must be briefly indicated, for by this means their

specificity is determined. Of course, many of the essential points in the digestion of proteins (by proteolytic ferments) have long been known. Nevertheless, the modern research with purified enzyme-preparations has added considerably to the older knowledge, and has aided materially in interpreting the older results.

It may today be accepted as proved that none of the known proteins are attacked by peptidases of either plant or animal origin (see table 2). In regard to their digestion by proteinases, however, one class of proteins occupies an exceptional position. These proteins are the protamines of fish-sperm, bodies of comparatively simple composition and strongly basic character (36). They are characterized by their inertness toward pepsinases, and their reactivity toward the tryptase-like protaminase (37). It is still undecided whether this peculiar behavior is due to their smaller molecular size (37), or to their simpler composition. Aside from the protamines, the proteins are not decomposed by protaminase.

The remaining digestible proteins, on the other hand, are split by pepsinases, papainases and by pancreas-trypsin. The enzyme action is in these three cases not identical, because in each case the decomposition products are different. As far as we know, the effect of the pepsinase is to split proteins into higher peptides, the papainase forms peptides of low molecular weight, and the pancreas-trypsin produces considerable quantities of amino-acids.

From table 2 may also be seen that an enzymic splitting of animal skeletal substances, among them silk-fibroin and the keratins, has never been observed. One may conclude then that the proteins of this group possess a characteristic structure, corresponding to their particular physiological function.

The investigation of the specificity of the peptidases has been of much greater significance. The experiments with synthetic peptides and peptide-derivatives have increased our knowledge of the mechanism of proteolytic processes, and of enzyme reactions in general. Whether a peptide is split by the amino-polypeptidase or by the dipeptidase is determined by the length of the peptide-chain (38), but in the case of the carboxy-polypeptidase, it is also dependent upon the nature of the component amino-acids. The difference in specificity between the amino- and carboxy-polypeptidases is therefore a question of the composition of the peptide, as well as of the length of the peptide chain (39).

As is shown by table 3, the dipeptides are for the most part specific substrates of the dipeptidase. They are not attacked by amino-polypeptidase in any case, and only by carboxy-polypeptidase in a few in-

stances where the peptide is characterized by a particular amino-acid content. The scission of the dipeptides phenylalanyl-arginine (40) and glutaminyl-tyrosine (41) by carboxy-polypeptidase is to be referred to the presence of the particular amino-acids arginine and tyrosine. The special influence of certain amino-acids is clearly seen by comparing the reactivity of tyrosine-free with that of tyrosine-containing polypeptides. An increase in the length of peptides like glycyl-glycine and leucyl-glycine, brought about by merely adding glycine components to the molecule, does not cause the peptides to react with carboxy-poly-

TABLE 3

Specific hydrolysis of peptides

(+ = hydrolysis; − = no hydrolysis)

	DIPEPTIDASE	AMINO-POLYPEPTIDASE	CARBOXY-POLYPEPTIDASE
Dipeptides:			
Glycyl-glycine	+	−	−
Leucyl-glycine	+	−	−
Glycyl-tyrosine	+	−	−
Histidyl-glycine	+	−	−
Phenylalanyl-arginine	+	−	+
Glutaminyl-tyrosine	+	−	+
Polypeptides:			
Leucyl-triglycine	−	+	−
Leucyl-triglycyl-leucyl-triglycyl-leucyl-triglycyl-leucyl-pentaglycine	−	+	−
Leucyl-glycyl-tyrosine	−	+	+
Leucyl-diglycyl-tyrosine	−	+	+
Leucyl-triglycyl-tyrosine	−	−	+

peptidase. The introduction of a tyrosine-residue, on the other hand, does, for as the table shows, the tri-peptide leucyl-glycyl-tyrosine, the tetra-peptide leucyl-diglycyl-tyrosine and the penta-peptide leucyl-triglycyl-tyrosine are split by carboxy-polypeptidase. Of these, the tyrosine-containing penta-peptide furnishes a really specific substrate for the ferment, since it is no longer attacked by the amino-polypeptidase, though the tri- and tetra-peptides are. The modes of action of the two enzymes are therefore not the same. It may be farther shown that the action of the carboxy-polypeptidase involves the carboxyl end

of the molecule, and stops with the splitting-off of the tyrosine, while the hydrolysis through amino-polypeptidase takes place at the end containing the free-amino-group. The hydrolysis of peptides by carboxy-polypeptidase is caused by the presence of several other amino-acids besides tyrosine. According to E. Abderhalden (42) cystine and tryptophane function similarly.

These results naturally bring up the question of the deeper causes of the differences in specificity between proteolytic enzymes. Today this question is in the way of being answered, thanks to the work done on the specific scissability of peptide-derivatives. There are distinct structural requirements for the scission of a peptide by means of any particular enzyme. These structural features of the peptide molecule are necessary for the action of the enzyme, because through them the combination between enzyme and substrate takes place (43).

The question of the nature of these enzyme-binding groups in the substrate was first solved in the case of the peptidases. The dipeptidase and the amino-polypeptidase attach themselves to the free amino-group of the peptide. Naturally, then, for these enzymes, the presence of a free amino-group in the substrate is a necessity. If it be altered, for instance by the introduction of an acyl group, the enzymes can no longer function. The free carboxyl-group of the peptide, however, may be removed without stopping the enzyme action (44). The effect of the amino-polypeptidase and of the dipeptidase on the peptide substrate consists in splitting off the amino-acid which originally carried the free amino-group. The difference in specificity between the amino-polypeptidase and the dipeptidase, and for which the length of the peptide chain is responsible, appears to be based upon differences in the acid character of the reacting groups between polypeptides and dipeptides. These differences naturally exert an influence upon the formation of the enzyme-substrate compound (45).

The union between the carboxy-polypeptidase and its subtrate is of quite another character. This enzyme unites with the carboxyl-group (46), and its action consists in splitting off the amino-acid carrying the free carboxyl-group of the peptide. The difference between the two types of enzyme-substrate combination, characteristic of the two polypeptidases, is most strikingly illustrated by the fact that compound-formation with the amino-polypeptidase is affected only by the steric configuration of the amino end of the molecule, while for compound-formation with the carboxy-polypeptidase only the steric configuration of the carboxyl end comes in question (47). Accordingly, the hydrolysis

of the racemic tripeptide d, l-leucyl-glycyl-l-tyrosine is asymmetric with the amino-polypeptidase, and is limited to splitting off the l-leucine, but with carboxy-polypeptidase the hydrolysis is symmetric, leading to the formation of l-tyrosine and d, l-leucyl-glycine.

The enzyme-binding groups in the peptides are, therefore, for the dipeptidase and the amino-polypeptidase, the NH_2-group, and for the carboxy-polypeptidase, the COOH-group. What, however, is the nature of the active groups of the enzyme molecule, its haptophore groups? Some light has been shed on this subject by investigating the variation of the enzyme-action with the hydrogen-ion concentration. For the case of the dipeptidase it has been shown that the variation of enzyme-action with pH corresponds exactly to the variation observed with pH in the case of a simple chemical reaction of the peptides, namely, their condensation with glucose. The form of the pH-curve is the same for the reaction of dipeptide with glucose as for the reaction of dipeptide with dipeptidase (48). Furthermore the pH influences the equilibrium point of the reaction between dipeptide and enzyme, but does not effect the decomposition-velocity of the enzyme-substrate compound (49). The conclusion is justified that in the reactions dipeptide-glucose and dipeptide-enzyme, similar chemically active groups take part. As H. v. Euler and K. Josephson (50) first suggested on the basis of inhibition experiments, the haptophore group of the dipeptidase (and also very probably of the amino-polypeptidase) might be considered an aldehyde or keto group. The anchorage of the peptidases on the peptide-NH_2 may quite well occur through the formation of a Schiff's base.

Our information about the haptophore group of the carboxy-polypeptidase is less definite. The anchorage of the enzyme on the carboxyl-group seems to require a certain degree of ionization of the COOH (51). The previously mentioned influence of special amino-acids like tyrosine on the digestibility of peptides by carboxy-polypeptidase seems referable to the electrochemical nature of the carboxyl-group. This conclusion follows from the observation that any peptide becomes digestible through carboxy-polypeptidase if an acid radical be introduced into the free amino-group (51). So, while diglycine is not attacked by the enzyme, benzoyl-diglycine is hydrolysed. The increased electro-negative character of the carboxyl, so produced, enables the enzyme to combine with it. The reaction of the carboxy-polypeptidase with its substrates, appears therefore to be an ionic reaction (52), and the haptophore group of the enzyme is most likely of basic nature.

The union of the ferment and substrate, that is, the formation of the

enzyme-substrate compound, does not complete the enzymic catalysis. It must be supplemented by the decomposition of the enzyme-substrate complex into free enzyme and the split-products of the substrate (53). There are different ideas concerning the mechanism of this decomposition.

E. Abderhalden (54) has lately offered the explanation (in the case of the dipeptidase) that the hydrogen-ion of the solution effects the decomposition of the peptide-peptidase compound. He believes that the CO—NH—linkage in the complex is more easily hydrolysed by hydrogen-ion, than is the linkage of the original peptide. This explanation, which is based upon the behavior of acylated peptides towards alkali, seems hardly sufficient. As stated above, it has been shown that the influence of the hydrogen-ion concentration upon the action of dipeptidase is confined to affecting the equilibrium between free and substrate-bound enzymes, and does not affect the decomposition of the enzyme-substrate complex.

Another explanation, today at least, more defensible, makes the assumption of more than one union between enzyme and substrate, and so corresponds to the "two-affinities-theory" of H. v. Euler. Experiments have shown that the specific action of dipeptidase is dependent not only upon the optical configuration of the amino-acid carrying the free amino-group (where the enzyme is known to attach itself), but also upon the optical configuration of the amino-acid carrying the carboxyl-group. For example, in the racemic glycyl-d, l-alanine, only the naturally occurring glycyl-d-alanine is hydrolysed. The existence in the peptide molecule of an atomic grouping which takes part in the decomposition of the peptide-peptidase complex seems probable in view of the experiments of A. K. Balls and Fr. Köhler (55). According to Balls and Köhler, the second point of attachment, not only for the dipeptidase, but for the amino- and carboxy-polypeptidases as well, is the imino-group of the subsequently severed peptide-linkage. This conclusion is reached by consideration of the hydrolysis of a series of substituted glycyl-anilines, so-called "aniline-peptides," and is corroborated by the behavior of other substituted acid-amides as inhibitory substances. All the peptides have this in common, then, that they unite with the imino-group of the peptide bond which is split. They differ, however, according to the other group in the substrate with which they combine. In the particular case of the dipeptidase, it seems evident that in addition a definite electro-negative character of the imino-group is necessary. This observation is corroborated by the greatly increased instability

shown by peptides in which the imino-hydrogen has been substituted by an acid radical (56). If the imino-hydrogen of a dipeptide is acetylated, the resulting substance decomposes spontaneously in weakly alkaline aqueous solutions, into its constituent amino-acids.

By these experiments on the mechanism of peptidase action, the "two-affinities-theory" has shown itself to be a valuable working hypothesis. It permits the explanation of enzyme action as an ordinary chemical reaction between definite chemical groups in enzyme and substrate, the variations in whose chemical (or electrochemical) affinities account for the phenomenon of enzymic specificity.

The general conception of enzyme action as a chemical rather than a surface phenomenon seems essentially well-grounded. The reactions of the enzymes, as well as their adsorptive behaviour, depends upon the action of specific chemical valencies.

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